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IL28B Genetic Variation and Cytomegalovirus-Specific T-Cell Immunity in Allogeneic Stem Cell Transplant Recipients

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A single nucleotide polymorphism (SNP), 3 kbp upstream of the IL28B gene (rs12979860; C/T), has been shown to influence the dynamics of cytomegalovirus (CMV) replication in allogeneic stem cell transplant recipients (Allo-SCT). We investigated whether this SNP had any effect on the dynamics of CMV-specific T-cell immunity in these patients. CMV pp65/IE-1 IFN-γ $CD8^+$ and $CD4^+$ T cells were enumerated by flow cytometry in 85 patients with no prior CMV DNAemia (group A) and in 57 after the onset of CMV DNAemia (group B). Donor IL28B genotype was determined by real-time PCR and plasma levels of IL-28B were quantitated by ELISA. CMV-specific T-cell counts and plasma IL-28B levels in patients in group A were not significantly different among the IL28B genotype groups. Patients harboring the donor IL28B T/T genotype appeared to expand CMV-specific IFN-γ $CD8^+$ cells to a higher level in response to viral replication than their C/T and C/C counterparts. Fewer patients in the T/T group received pre-emptive antiviral therapy ($P = 0.05$). Overall, a significant inverse correlation was observed between median IL-28B levels measured prior to the CMV DNAemia onset and the level of CMV-specific $CD8^+$ T cells enumerated after detection of CMV DNAemia ($\sigma = -0.471$; $P = 0.013$). In summary, the data suggested that the protective effect attributed to the rs12979860 SNP minor T allele could be mediated, at least in part, by eliciting robust CMV-specific T-cell responses. ***J. Med. Virol. 89:685–695, 2017***.

KEY WORDS: cytomegalovirus; allogeneic stem cell transplantation IFN λ3; IL28B gene polymorphisms; T-cell immunity; CMV DNAemia

INTRODUCTION

Cytomegalovirus (CMV) infection is a relevant cause of morbidity and mortality in allogeneic stem cell transplant recipients (Allo-SCT) [Boeckh and Ljungman, 2009; Pérez-Romero et al., 2015]. The impact of host and donor genetics on the risk of active CMV infection and end-organ disease in Allo-SCT patients is being increasingly recognized [Loeffler et al., 2006; Mezger et al., 2008; Bravo et al., 2014; Corrales et al., 2015]. Type III (λ) interferons (IFNs), including IFN-λ1 (IL-29), IFN-λ2 (IL-28A), and IFN-λ3 (IL-28B) and IFN-λ4, are triggered by viral infections and may induce antiviral activity, which may partially overlap with that mediated by type I IFNs, as well as immunomodulatory effects [Kotenko et al., 2003; Sheppard et al., 2003], as they signal through a

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heterodimeric receptor consisting of IL-10Rβ and IL-28R1 chains. The antiviral effect against a wide variety of viruses, including influenza virus, human metapneumovirus, herpes simplex virus type 2, respiratory syncytial virus and rotavirus has been demonstrated in experimental models [Hermant and Michiels, 2014]. Virtually any cell type is capable of expressing IL28B mRNA; Nevertheless, IL-28B is mainly produced by myeloid and plasmacytoid dendritic cells [Yoshio et al., 2013; Zhang et al., 2013]. IL-28B binds a specific cell surface receptor complex which is abundantly expressed in epithelial cells, melanocytes, and hepatocytes [Sommereyns et al, 2008], leading to JAK-STAT signal transduction and expression of interferon-stimulated genes [Liu et al., 2012]. A single nucleotide polymorphism (SNP; rs12979860), 3 kbp upstream of the IL28B gene, has been shown to be associated with spontaneous resolution of hepatitis C virus (HCV) infection and with a greater response of patients infected chronically with HCV genotype 1 to PEG-IFNa/Ribavirin therapy [Ge et al., 2009; Thomas et al., 2009]. This if one of a series of SNPs located relatively distant from the IL28B and IFNL4 coding sequences genes that have been shown to be in high linkage disequilibrium [Duggal et al., 2013]. Despite its location they are thought to potentially impact the binding of transcription factors and methylation sites, thereby influencing the promoter activity [Chinnaswamy et al., 2013; McFarland et al., 2014; Lu et al., 2015]. Nevertheless, the exact mechanism by which these SNP affect regulation or expression of the IL28B gene remains to be elucidated.

We previously investigated the effect of the IL28B SNP rs12979860 on the incidence of active CMV infection and the dynamics of CMV DNAemia within episodes of CMV replication in Allo-SCT recipients. Our data pointed to a protective effect of the donor T allele (just the opposite to that seen in HCV-infected patients). In particular, patients receiving an allograft from a donor harboring the minor allele expressed in homozygous (T/T) form had shorter episodes of CMV DNAemia than those carrying the donor C/T and C/C genotypes. In contrast, the recipient IL28B genotype had no influence on the variables subject to analysis. A relevant impact of the above-mentioned SNP on the incidence of active CMV infection in solid organ transplant recipients has also been reported [Egli et al., 2014; Fernández-Ruiz et al., 2015]. A plausible mechanistic explanation for these clinical observations was proposed by Egli et al. [2014], whereby lower CMV-induced interferon-stimulated genes (ISG) and an impairment of T-cell priming during CMV replication would be the hallmarks of the risk genotypes (C/C and C/T), this leading to a suboptimal antiviral response. In this context, we investigated whether the IL28B rs12979860 SNP had any effect on the recovery of CMV-specific T-cell immunity following transplantation and on the magnitude of CMV-specific T-cell expansion triggered by CMV replication in Allo-SCT recipients.

MATERIALS AND METHODS

Patients

The current study was a retrospective, observational one that included two independent cohorts: Cohort (A) consisted of 85 non-consecutive, Caucasian patients who underwent Allo-SCT at the respective Haematology Unit of the Hospital Clínico Universitario, Valencia, Spain (HCU) (n = 48), Hospital La Princesa, Madrid, Spain (n = 18), Hospital Ramón y Cajal, Madrid, Spain (n = 11), and Hospital Morales Meseguer, Murcia, Spain (n = 8) between 2008 and 2014. The inclusion criteria were the following: (i) absence of documented CMV DNAemia prior to the time of immunological analysis; (ii) availability of CMV-specific $CD8^+$ T-cell immunity data; (iii) availability of at least three plasma specimens for IL-28B quantitation obtained after Allo-SCT and prior to the time of immunological analyses; and (iv) availability of data on the donor IL28B genotype. Cohort (B) consisted of 57 non-consecutive patients who underwent Allo-SCT at HCU between 2007 and 2014. The inclusion criteria were the following: (i) availability of data on CMV-specific $CD8^+$ T-cell immunity obtained within the episode of active CMV infection; (ii) availability of plasma specimens for IL-28B quantitation obtained prior to the time of immunological analyses; and (iii) availability of data on the donor IL28B genotype. The patients in cohorts A and B had been included in different studies previously published by our group [Solano et al., 2008; Tormo et al., 2010a,b, 2011; Giménez et al., 2015a,b]. In all these studies, CMV-specific IFN-γ-producing $CD8^+$ T cells, but not $CD4^+$ T cells, were planned to be enumerated. As a result, the number of available measurements for CMV-specific $CD4^+$ T cells was lower than that for CMV-specific $CD8^+$ T cells. The donor IL28B genotype of 100 out of the 142 patients included in the current study was previously reported [Bravo et al., 2014]. The median age of patients included in the current study was 48.6 years (range 18–70 years). The study period comprised the first 100 days following transplantation. Only initial (first) episodes of CMV DNAemia were considered for the analyses described herein. The study was approved by the Ethics Committees of the participating hospitals. All patients gave their informed consent to participate in the study.

Virological Monitoring and Management of Active CMV Infection

Virological monitoring was conducted at least once a week, and was performed with a pp65 antigenaemia assay (AG) (Diagnostics CMV pp65 Antigenemia Immunofluorescence assay, Chemicon International, Temecula, CA) and/or by a plasma real-time PCR assay, as previously reported [Tormo et al., 2011]. For the AG assay, blood samples were obtained in EDTA-treated tubes and were processed within 2 hr.

Polymorphonuclear leukocytes (PMNLs) and plasma were separated by the standard dextran sedimentation method. The pp65 antigenemia assay was carried out by a standard immunofluorescence procedure as previously described [Solano et al., 2001]. Results were reported as the number of pp65-positive cells/200,000 PMNL. Pre-emptive therapy with oral valganciclovir (900 mg/12 hr), i.v. ganciclovir (5 mg/kg/12 hr), or i.v. foscarnet (60 mg/kg/12 hr) was initiated either upon a positive pp65 AG result or upon a CMV DNAemia threshold level, which was established at each center (between 500 and 1000 copies/ml). All patients in cohort B underwent Allo-SCT at the Hospital Clínico Universitario of Valencia. At this center, until April 2010, virological monitoring was performed in parallel with the AG assay and a real-time PCR (PCR Kit, Abbott Molecular, Des Plaines, IL), although the initiation and cessation of pre-emptive antiviral therapy was based exclusively upon AG assay results. After May 2010, virological monitoring was performed exclusively by real-time PCR (the new Abbott Real Time CMV assay replaced the old CMV PCR kit by May 2012). Plasma CMV DNAemia values were normalized to the 1st WHO International Standard for CMV for Nucleic Acid Amplification (NAT)-Based Assays (National Institute for Biological Standards and Control, Hertfortshire, UK), and reported in International Units (IU)/ml throughout the text. The conversion factor (copies/ml to IU/ml) for these real-time PCR assays was previously defined [Clari et al., 2013]. Real-time PCR assyays were performed following the instructions of the manufacturer.

IL28B Polymorphism Analysis

IL28B (rs12979860) genotyping was performed by PCR (LightMix Kit IL28B; TIB MOLBIOL, Berlin, Germany) using the Roche Diagnostics LightCycler 2.0 following the recommendations of the manufacturer (Insert LightMix® Kit IL28B rs12979860; Cat.-No. 40-0588-32). Briefly, a 139 bp long fragment is amplified with specific primers and analyzed in a subsequent melting curve analysis, using a SimpleProbe oligomer, which is specific for the −3176C allele. The resulting PCR fragments are detected by the SimpleProbe® in channel 530. The genotypes are identified by running a melting curve with specific melting points (Tm). The allele T exhibits a Tm of about 51–53°C. Plasma samples were obtained from patients following engraftment (collected around 30 days after Allo-SCT) to determine the donor genotype. It has been shown that cell-free DNA in plasma in Allo-SCT is predominantly of hematopoietic origin [Lui et al., 2002]. All patients had converted into full donor chimera at that time. DNA was extracted with the EZ1 Virus 2.0 Kit (Qiagen, Valencia, CA) on the BioRobot EZ1 extraction platform (Qiagen), following the instructions of the manufacturer. Plasma specimens had been cryopreserved at −70°C, and were retrieved for analysis.

Immunological Monitoring

Enumeration of CMV-specific interferon-gamma (IFN-γ)-producing $CD8^+$ and $CD4^+$ T cells was carried out by flow cytometry for intracellular cytokine staining-ICS- (BD Fastimmune, BD [Becton Dickinson and Company] Biosciences, San Jose, CA). Whole blood samples were processed within 24–30 hr after collection (in our experience, such a delay in specimen processing has no significant effect on the responsiveness of T cells to CMV). Heparinized whole blood specimens (0.5 ml) were simultaneously stimulated for 6 hr with two sets of 15-mer overlapping peptides encompassing the entire sequence of pp65 and IE-1 CMV proteins (1 μg/ml per peptide) in phosphate-buffered saline/dimethyl sulfoxide [PBS/DMSO]), obtained from JPT Peptide Technologies GmbH (Berlin, Germany), in the presence of 1 μg/ml of costimulatory mAbs to CD28 and CD49d. Samples mock stimulated with PBS/DMSO (without peptides) and costimulatory antibodies or stimulated with 1 mg/ml of phytohemagglutinin (Sigma–Aldrich, St. Louis, MO) were run in parallel. Brefeldin A (10 μg/ml) was added for the last 4 hr of incubation. Blood was then lysed and frozen at 80°C until tested. On the day of testing, stimulated blood was thawed at 37°C, washed, permeabilized, and stained with a combination of labeled monoclonal antibodies (anti-IFN-γ-FITC, anti-CD69-PE, anti-CD4, or CD8-PerCP-Cy5.5, and anti-CD3-APC, when the IFN-γ $CD8^+$ kit was used) for 30 min at room temperature. In some experiments, anti-CD3–APC—Cy7 (BD, Biosciences) was used instead of anti-CD3-APC [Giménez et al., 2015a,b]. Appropriate isotype controls were used (BD FastImmune TMc2a/c1/CD8/CD3 reacting with keyhole limpet hemocyanin; BD Biosciences). Cells were then washed, resuspended in 200 μl of 1% paraformaldehyde in PBS, and analyzed within 2 hr on a FACSCalibur flow cytometer using CellQuest software (BD Biosciences Immunocytometry Systems, San Jose, CA). Cells were first gated for lymphocytes (SSC-A vs. FSC-A). The lymphocyte population was analyzed for their surface expression of CD3 and CD8 (or CD4). CD3/CD8- (or CD4-) expressing cells were further gated for their expression of the activation marker CD69 and the intracellular cytokine IFN-γ (see Fig. S1, supplementary material). Cells were analyzed within 2–4 hr of staining on a FACSCalibur flow cytometer using CellQuest software (BD Biosciences Immunocytometry Systems) or on a BD FACScantoII flow cytometer (BD) and analyzed using the programs BD FACS Diva Software (BD Biosciences) and Infinicyt 1.4/1.7 (Cytognos). Data files usually contained at least 1,000 positive events for $CD4^+$ or $CD8^+$ within the lymphocyte gate. The total number of CMV-specific $CD4^+$ and $CD8^+$ T cells was calculated by multiplying the percentages of CMV-specific T cells producing IFN-γ on stimulation (after background subtraction) by the absolute $CD4^+$ and $CD8^+$ T-cell counts. A detectable response of >0.1%

(two standard deviations higher than the median of CMV-seronegative individuals) was counted as positive.

IL-28B Measurements

Plasma specimens for IL-28B measurements had been obtained within 2 hr upon reception of blood (samples were centrifuged at 2,000 rpm for 10 min). The plasma fraction was transferred to a new tube and was aliquoted in small, single-use volumes to avoid multiple freeze-thaw cycles. These samples were stored at −80°C until use. Plasma IL-28B levels were determined with an ELISA kit specific for IL-28B (Human IL-28B, Shangai SunRed Biological Technology, Shanghai, China). The ELISA was carried out strictly following the manufacturer's instructions. The sensitivity of the assay is 0.68 pg/ml and the linear range of quantitation is between 0.8 and 2000 pg/ml. According to the manufacturer and to our own observations (data not shown), the intra- and interassay coefficients of variation are <10%. Optical density was determined using a microtiter plate reader at 450 nm. All analyses and calibrations were performed in duplicate (mean values were considered for analyses). Long-term storage (up to 2 years) had no effect on IL-28B measurable levels (data not shown).

Statistical Analyses

Frequency comparisons were carried out using the χ^2 test (Fisher's exact test) for categorical variables. Differences between medians were compared using the Mann–Whitney U-test (two independent variables). The correlation between continuous variables was established by the Spearman Rank test. Two-sided exact P values are reported. A P-value <0.05 was considered to indicate statistical significance. The IL28B SNP was in Hardy–Weinberg equilibrium in the study groups, as determined by a χ^2 test using 1° of freedom [Bravo et al., 2014]. Pairwise comparisons among the different IL28B genotype groups for each parameter were performed. Data were analyzed with the aid of the statistical package SPSS version 20.0 (SPSS).

RESULTS

Donor IL28B Genotypes and CMV-Specific T-Cell Levels in Allogeneic Stem Transplant Recipients

We first investigated whether the level of CMV-specific T cells measured early after transplant differed among the donor IL28B rs12979860 SNP genotype groups. We selected a total of 85 patients who had not documented CMV DNAemia prior to the time of the first immunological analysis (performed around day +30) (cohort A). Patients with a preceding episode of CMV DNAemia were excluded deliberately, as CMV replication triggers CMV-specific T-cell expansion, whose magnitude may differ substantially between Allo-SCT recipients. Thirty-six of these patients harbored the donor IL28BC/C genotype, 39 the C/T genotype and 10 were homozygous for the minor T allele. There were no significant differences in the clinical and demographic characteristics of patients between the different donor IL28B genotype groups (Table I). The age of patients was also comparable between groups (C/C, median, 51.1 years, C/T, median 51.0 years, and T/T, median 47.5 years; $P = 0.473$). In this group, a trend towards a lower incidence of CMV DNAemia was observed for patients carrying the T/T genotype (10% vs. 26% for C/T and C/C patients; $P = 0.262$). It must be stressed that the incidence of CMV DNAemia in this study group did not reflect the actual incidence of active CMV infection in Allo-SCT patients (much higher), as patients with CMV DNAemia occurring earlier than the time of immunological monitoring (day +30 in most patients) were not taken into account for this analysis. Peripheral CMV-specific IFN-γ $CD8^+$ T-cell counts (total number) were available for all 85 patients. Only 42 patients (donor genotypes: 15C/C, 23C/T, and 4T/T) had CMV-specific IFN-γ $CD4^+$ T-cell counts available for analysis. Detectable CMV-specific IFN-γ $CD8^+$ T-cell responses were observed

TABLE I. Demographic and Clinical Characteristics of Patients Included in Cohort A According to the Donor IL28B Genotype

	IL28B genotype (no. of patients)			
Parameter	C/C	C/T	T/T	P-value*
Sex				0.095
Female	17	11	6	
Male	19	28	4	
Type of transplant				0.845
Peripheral blood	32	36	10	
Umbilical cord blood	2	1	0	
Bone marrow	2	2	0	
Type of donor				0.131
Related	26	17	7	
Unrelated	10	22	3	
HLA matching				0.568
HLA-matched	33	31	9	
HLA-mismatched	3	7	1	
Unknown	0	1	0	
CMV serology				0.885
D+/R+	22	24	6	
D−/R+	8	12	3	
D−/R−	1	1	0	
D+/R−	5	2	1	
Conditioning regimen				0.481
Myeloablative	22	20	8	
No myeloablative	14	19	2	
Acute graft versus host disease				0.143
Grades 0–I	28	29	5	
Grades II–IV	8	10	5	

D, donor; R, recipient.
*χ^2 test.

more frequently in T/T patients (90%) than in their C/T and C/C counterparts (77%), although the difference did not reach statistical significance ($P = 0.525$). In turn, detectable CMV-specific IFN-γ $CD4^+$ T-cell responses were observed at a rather similar frequency for all IL28B genotype groups (75% for TT patients and 78% for C/T plus C/C patients; $P = 0.634$). As shown in Figure 1, median peripheral levels of CMV-specific IFN-γ $CD8^+$ and $CD4^+$ T cells were not significantly different between the comparative groups.

Plasma Levels of IL-28B According to the Donor IL28B Genotype

A total of 50 out of the 85 patients in cohort A had at least three plasma specimens (median 4; range 3–6) obtained prior to the time of immunological monitoring available for IL-28B quantitation (C/C, n = 19; C/T, n = 26 and T/T, n = 5). Overall, plasma IL-28B levels fluctuated over this period of time, although no specific trend was associated with any particular IL28B genotype (not shown). Both the median IL28B level of all measurements per patient (Fig. 2A) and the peak IL-28B level in these specimens (Fig. 2B) were comparable between the IL28B genotype groups. Likewise, no correlation was found between the peak IL-28B levels and the magnitude of CMV-specific $CD8^+$ (Fig. 3A) or $CD4^+$ T-cell (3B) responses.

CMV-Specific IFN-γ T Cells After the Onset of CMV DNAemia According to the IL28B Genotype

We next investigated whether the IL28B rs12979860 SNP had any effect on the magnitude of the CMV-specific IFN-γ $CD8^+$ and $CD4^+$ (when available) T-cell responses triggered by CMV replication. Out of the 57 patients included in this cohort (B), 25 harbored the donor C/C genotype, 23 the C/T genotype, and 9 the T/T genotype. The three groups did not differ significantly in terms of (i) age (C/C, median, 52 years; C/T, median 55 years; T/T, median 48 years; $P = 0.631$); (ii) demographic and clinical pre- and post-transplant factors known to modulate the susceptibility to active CMV infection and end-organ disease (Table II); (iii) The magnitude of the CMV DNA load at the onset of CMV DNAemia ($P = 0.560$) (Fig. 4A). This is of relevance, as the level of CMV replication has been shown to influence the extent to which CMV-specific IFN-γ $CD8^+$ T cells expand [Hakki et al., 2003; Tormo et al., 2010a; 2011; Guerrero et al., 2012]; or (iv) the time elapsed between the onset of CMV DNAemia and the moment at which immunological monitoring was performed (median of around 20 days in all groups; $P = 0.472$). The data indicated that the patients harboring the donor IL28B T/T genotype displayed CMV-specific IFN-γ $CD8^+$ T-cell responses of higher magnitude (median, 1.79 cells/μl) than their C/T and C/C counterparts (median, 0.45 and 0.19 cells/μl, respectively) (Fig. 5A). The difference, however, did not reach statistical significance. This trend, however, was not observed for CMV-specific $CD4^+$ T cells (Fig. 5B). In relation to this observation, we found that the plasma CMV DNA peak level during episodes of active CMV infection was substantially lower in patients harboring the IL28B T/T genotype than in the latter group, although statistical significance was not reached ($P = 0.118$) (Fig. 4B). As a result, fewer patients in the T/T group received pre-emptive antiviral therapy (33% in the T/T group, 65.2% in the C/T group, and 80% in the C/C group; $P = 0.05$).

Plasma Levels of IL-28B and CMV-Specific T-Cell Responses in Patients With CMV DNAemia

Plasma specimens obtained prior (one specimen per patient) and during (two specimens per patient) the episode of active CMV infection were available for 27 patients (12C/C, 13C/T, and 2T/T) in group B. All of

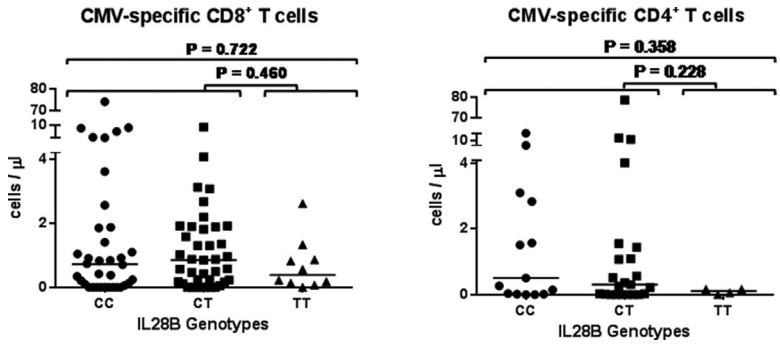

Fig. 1. CMV-specific T-cell responses measured in Allogeneic stem cell transplant recipients with no prior CMV DNAemia according to the donor IL28B (rs12979860) genotype. Peripheral CMV pp65/IE-1-specific IFN-γ-producing $CD8^+$ and $CD4^+$ T cells total counts (one determination/patient) were available from 85 patients (group A). In these patients, immunological data were obtained on day +30 (n = 67), between days +30 and +50 (n = 13), or on day +60 (n = 5). Only 42 patients had CMV-specific IFN-γ $CD4^+$ T-cell counts available for analysis (data obtained on day +30, n = 37 patients, and between days +30 and +50, n = 5 patients). Bars indicate median values. Differences between medians were compared using the Mann–Whitney U-test. *P* values for statistical comparisons between groups are shown (TT vs. CT vs. CC, upper bracket; TT vs. C/T plus C/C, lower bracket).

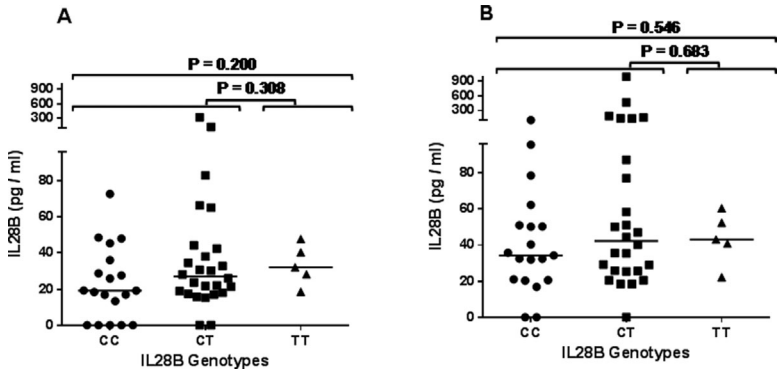

Fig. 2. Plasma IL-28B levels in Allogeneic stem cell transplant recipients with no prior CMV DNAemia according to the donor IL28B (rs12979860) genotype. Plasma IL-28B levels (mean of duplicates) were measured in 50 Allo-SCT patients (cohort A) who had no CMV DNAemia prior to the time of the first immunological analysis (performed around day +30 after transplant). Median values of at least three plasma specimens per patient, obtained between days 7 and 42 after transplant, are shown in panel **A**. Median values of IL-28B peak concentrations found in these plasma specimens are shown in panel **B**. Bars indicate median values. Differences between medians were compared using the Mann–Whitney U-test. *P* values for statistical comparisons between groups are shown (TT vs. CT vs. CC, upper bracket; TT vs. C/T plus C/C, lower bracket).

these samples were drawn prior to the time of immunological monitoring. CMV-specific IFN-γ-$CD8^+$ and $CD4^+$ T-cell counts were available from 27 and 23 of these patients, respectively, and were enumerated a median of 21 days after detection of CMV DNAemia. The low number of patients in the IL28B T/T group precluded any meaningful analysis via direct comparisons of plasma levels of IL-28B between the three groups. Nevertheless, we investigated whether there was an overall correlation, irrespective of IL28B genotype, between plasma levels of IL-28B and CMV-specific T-cell numbers. A significant inverse correlation was found between IL-28B plasma levels measured prior to the occurrence of CMV DNAemia and CMV-specific $CD8^+$, but not $CD4^+$, T-cell levels enumerated after CMV DNAemia onset (Fig. 6). Likewise, a trend towards an inverse correlation between plasma IL-28 levels measured after detection of CMV DNAemia and CMV-specific $CD8^+$ and $CD4^+$ T cells was observed, although statistical significance was not reached (Fig. 7).

DISCUSSION

An increasing body of clinical and experimental evidence indicates that certain polymorphisms located near or within the IL28B gene may modulate the risk of active CMV infection and the dynamics of CMV replication in the transplantation setting [Bravo et al., 2014; Egli et al., 2014; Fernández-Ruiz et al., 2015; Manuel et al., 2015]. In a prior study, we showed a trend towards a lower incidence of episodes of active CMV infection developing within 100 days after Allo-SCT in patients carrying the homozygous TT genotype of the IL28B (rs12979860) SNP in comparison with their C/C and C/T counterparts [Bravo et al., 2014]. Likewise, we reported that patients homozygous for the T allele had episodes of

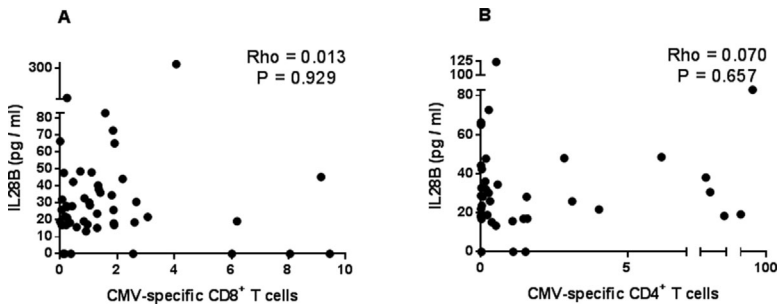

Fig. 3. Correlation between plasma IL-28B levels and CMV-specific T-cell levels in patients with no prior CMV DNAemia irrespective to the donor IL28B genotype. Plasma IL-28B levels (mean of duplicates) were measured in 50 Allo-SCT patients (cohort A) who had no CMV DNAemia prior to the time of the first immunological analysis. Median values of at least three plasma specimens per patient are shown. Correlations of plasma IL-28B levels with CMV pp65/IE-1 $CD8^+$ (panel **A**) and $CD4^+$ (panel **B**) T-cell levels are shown. Immunological data (a single blood sample per patient) were obtained on day +30 (n = 67), between days +30 and +50 (n = 13), or on day +60 (n = 5). Only 42 patients had CMV-specific IFN-γ $CD4^+$ T-cell counts available for analysis. The correlation between these parameters was measured by the Spearman Rank test. Two-sided exact *P* values are shown.

TABLE II. Demographic and Clinical Characteristics of Patients Included in Cohort B According to the Donor IL28B Genotype

Parameter	IL28B genotype (no. of patients) C/C	C/T	T/T	*P*-value*
Sex				0.701
Female	11	9	5	
Male	14	14	4	
Type of transplant				0.420
Peripheral blood	18	19	6	
Umbilical cord blood	6	2	3	
Bone marrow	1	2	0	
Donor				
Related	12	13	5	0.825
Unrelated	13	10	4	
HLA-matching				
HLA-matched	16	17	7	0.651
HLA-mismatched	9	6	2	
CMV serology				0.613
D+/R+	16	15	8	
D−/R+	8	7	1	
D+/R−	1	1	0	
Conditioning				0.056
Myeloablative	9	5	6	
No myeloablative	16	18	3	
Acute graft versus host disease				0.499
Grades 0–I	17	12	6	
Grades II–IV	8	11	3	

D, donor; R, recipient.
*χ^2 test.

active CMV infection significantly shorter in duration than those displaying the C allele in homozygous or heterozygous form. Thus the data pointed to a protective effect of the T allele against CMV infection.

A decreased incidence of active CMV infection has also been reported to occur in high-risk SOT patients after suspension of antiviral prophylaxis and in CMV-seropositive kidney recipients managed by pre-emptive antiviral therapy strategies and carrying the IL28B T/T genotype [Egli et al., 2014; Fernández-Ruiz et al., 2015]. Likewise, Manuel et al. [2015] reported that a functional TT/-G polymorphism (rs368234815) in the CpG region upstream of IL28B influences susceptibility to CMV replication in SOT recipients not receiving antiviral prophylaxis.

Egli et al. [2014] reported that IL-28B impairs priming of the adaptive immune response to CMV. In line with this, recombinant IFN-λ was shown to promote the expansion of Foxp3$^+$ regulatory T cells [Dolganiuc et al., 2012] in vitro, which may be detrimental to the control of CMV replication [Egli et al., 2012].

In this context, the current work was aimed at determining whether the donor IL28B (rs12979860) SNP had any impact on the magnitude of CMV-specific T-cell response in Allo-SCT recipients. We were first interested in investigating whether this SNP had any influence on the level of CMV-specific T cells measured early after transplant (around day +30). To this end, we selected a cohort of 85 patients who displayed no evidence of CMV DNAemia prior to immunological monitoring and enumerated pp65/IE-1-specific IFN-γ-producing CD8$^+$ and CD4$^+$ T cells, as the peripheral blood levels of these functional T-cell populations have been shown to correlate closely with protection from CMV viremia [Solano et al., 2008; Tormo et al., 2011].

In this cohort, a trend toward a lower incidence of CMV DNAemia was observed in patients with the IL28B T/T genotype. Interestingly, detectable CMV-specific IFN-γ CD8$^+$ but not CD4$^+$ T-cell responses were observed more frequently in T/T patients than in their C/T and C/C counterparts, although median levels of peripheral CMV-specific IFN-γ CD8$^+$ T-cell precursors were not significantly different between the comparative groups. Also of interest was the fact that both the median and peak plasma IL-28B levels measured prior to the first immunological analysis did not differ significantly between the different IL28B genotype groups.

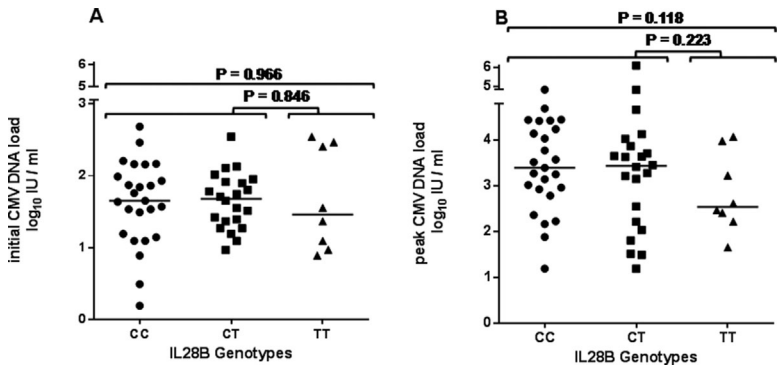

Fig. 4. Virological features CMV DNAemia episodes. Initial CMV DNA load (panel **A**) and CMV DNA peak load (panel **B**) of CMV DNAemia episodes developing in patients in cohort B are shown. Bars indicate median values. Differences between medians were compared using the Mann–Whitney U-test. *P* values for statistical comparisons between groups are shown (TT vs. CT vs. CC, upper bracket; TT vs. C/T plus C/C, lower bracket).

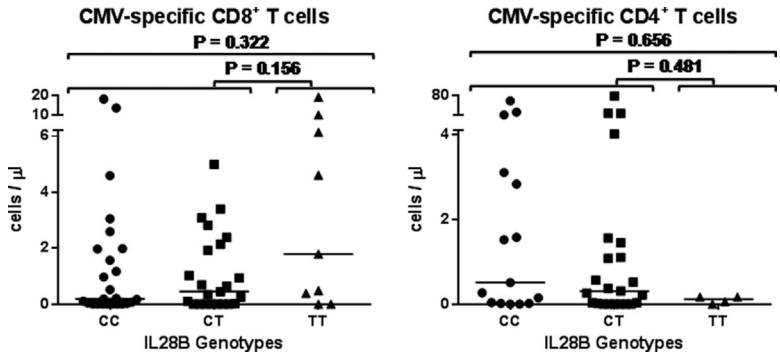

Fig. 5. CMV-specific IFN-γ T cells after the onset of CMV DNAemia according to the IL28B genotype. CMV pp65/IE-1 $CD8^+$ and $CD4^+$ T cells were enumerated in 57 patients—cohort B—(a single determination/patient) a median of around 20 days after CMV DNAemia onset in all groups. CMV-specific $CD8^+$ T-cell counts were available for all patients, whereas CMV-specific $CD4^+$ T-cell counts were available only for 27 patients. Bars indicate median values. Differences between medians were compared using the Mann–Whitney U-test. *P* values for statistical comparisons between groups are shown (TT vs. CT vs. CC, upper bracket; TT vs. C/T plus C/C, lower bracket).

We were next interested in determining whether the magnitude of expansion of CMV-specific T cells triggered by CMV replication varied according to the IL28B rs12979860 SNP genotype. To address this issue, we selected a cohort of 57 patients who had an episode of active CMV infection prior to immunological monitoring (group B). In these patients, enumeration of CMV-specific T cells was performed around 20 days after the onset of CMV DNAemia. Our data indicated that patients harboring the IL28B T/T genotype appeared to expand CMV-specific IFN-γ $CD8^+$ T cells to a higher level than their C/T and C/C counterparts. This despite the fact that the magnitude of the CMV DNA load at the onset of CMV DNAemia was comparable between groups. In keeping with these observations, we found that plasma CMV DNA peak levels during episodes of active CMV infection were markedly lower in patients harboring the IL28B T/T genotype than in the latter groups, and that pre-emptive antiviral therapy was less likely to be prescribed to IL28B T/T patients.

In patients in group B, a significant inverse correlation between plasma IL-28B levels measured prior to the time of detection of CMV DNAemia (and the time of immunological monitoring) and CMV-specific $CD8^+$ T-cell levels was observed. Likewise, a trend towards an inverse correlation between plasma IL-28 levels measured after detection of CMV DNAemia and CMV-specific $CD8^+$ and $CD4^+$ T cells was noted. In contrast, no correlation was found between IL-28B levels and CMV-specific T-cell responses in patients with no documented viremia. Although speculative, these data suggested that the detrimental effect of IL-28B on the activation and expansion of CMV-specific $CD8^+$ T cells may only be evident in the context of high-level CMV replication episodes in which the virus enters the blood compartment.

There is currently not a conclusive mechanistic explanation accounting for the apparent protective effect of the SNP (rs12979860) T allele against CMV infection in transplant patients. In our hypothesis, patients harboring the T allele in homozygous would be capable of generating stronger functional

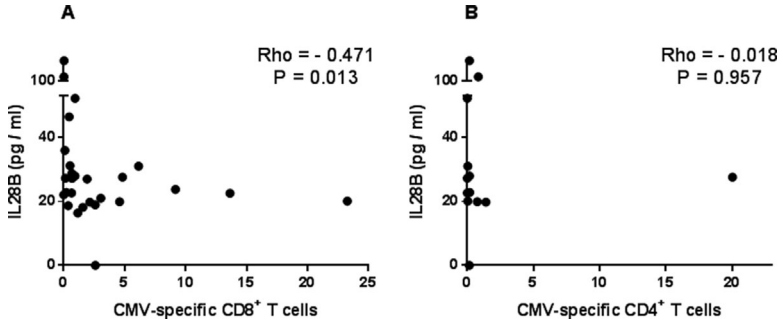

Fig. 6. Correlation between pre-CMV DNAemia plasma IL-28B levels and CMV-specific T-cell responses measured after CMV DNAemia onset. (**A**) CMV-specific $CD8^+$ T cells and (**B**) CMV-specific $CD4^+$ T cells. Plasma specimens obtained prior the episode of CMV DNAemia (one specimen per patient) were available for 27 patients in group B. All of these samples were drawn prior to the time of immunological monitoring. CMV-specific IFN-γ-$CD8^+$ and $CD4^+$ T-cell counts were available from 27 and 23 of these patients, respectively, and were obtained a median of 21 days after detection of CMV DNAemia. The correlation between these parameters was measured by the Spearman Rank test. Two-sided exact *P* values are shown.

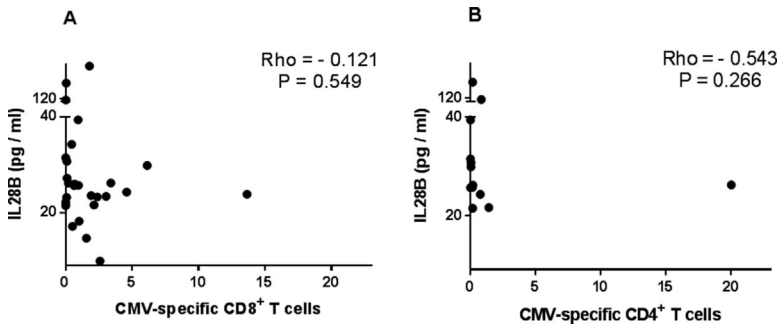

Fig. 7. Correlation between plasma IL-28B levels and CMV-specific T-cell responses measured after CMV DNAemia onset. (**A**) CMV-specific $CD8^+$ T cells and (**B**) CMV-specific $CD4^+$ T cells. Plasma specimens obtained after CMV DNAemia detection were available for 27 patients in group B. All of these samples were drawn prior to the time of immunological monitoring. Median levels of two determinations/patients are shown. CMV-specific IFN-γ-$CD8^+$ and $CD4^+$ T-cell counts were available from 27 and 23 of these patients, respectively, and were obtained a median of 21 days after detection of CMV DNAemia. The correlation between these parameters was measured by the Spearman Rank test. Two-sided exact *P* values are shown.

CMV-specific T-cell responses upon virus replication challenge than their C/T and C/C counterparts, perhaps either through a more efficient activation of antigen-presenting cells and T-cell priming, a limited expansion of Treg cells or both.

IL-28B signals signal through an heterodimeric receptor consisting of IL-28R1 chains and IL-10Rβ, thus inducing an antiviral state, which may partially overlap with that mediated by type I IFNs, as well as immunomodulatory effects [Kotenko et al., 2003; Sheppard et al., 2003]. In support of our hypothesis, pre-treatment of C/T fibroblasts with IL-28B led to a dose-dependent inhibitory effect on CMV-induced IFN-α2 mRNA expression and notably a marked suppressive effect on antiviral ISG expression. In contrast, anti-inflammatory ISGs (USP18 and SOCS1), remained upregulated during CMV infection [Egli et al., 2014]. Likewise, pre-treatment of fibroblasts with recombinant IL-28B blocked IFN-α2 induced STAT-2 phosphorylation [Egli et al., 2014]. In this sense, it is well known that the JAK-STAT signaling pathway regulates the expression of a wide array of genes involved in the activation of immune responses upon interaction with interferons and cytokines. We are currently planning to perform several "in vitro" experiments which may lend further support to our hypothesis. First, it seems crucial to demonstrate that the above observations, made in fibroblasts, can be reproduced in antigen-presenting cells. Second, activation and expansion of CMV-specific T cells and generation of Treg cells upon infection of peripheral blood mononuclear cells with CMV should be investigated in individuals harboring the T/T, C/T, or C/C IL28B SNP (rs12979860) genotypes.

The present study has several limitations that deserve comment. First, the relatively low number of patients carrying the donor IL28B (rs12979860) T/T genotype included in the study undermined the robustness of our statistical analyses. In fact, trends, rather than statistically significant associations, were found throughout these analyses. Although speculative, it is likely that more consistent data could have been obtained had we included more IL28B T/T patients. Unfortunately, given the scarce representation of the T/T genotype among Caucasians, recruiting a sufficient number of T/T patients was out of our reach. In this sense, a multicenter and adequately powered study is warranted to test our hypothesis. Second, the use of non-uniform strategies of pre-emptive antiviral therapy at the participating centers as well as the use of methods for virological monitoring displaying different sensitivities (pp65 antigenaemia and commercially available real-time PCR methods with different limits of detection and quantitation) could have influenced our observations. Third, due to the retrospective nature of our study, a number specimens were not available for immunological measurements. Fourth, the lack of follow-up specimens for determining precisely the kinetics of CMV-specific T-cell expansion after CMV DNAemia detection. Fifth, it is uncertain whether plasma levels of IL-28B correlate with those in organs and tissues. Likewise, the immunomodulatory effect of IL-28B may depend on the extent of expression and distribution of IFN-λ receptors in tissues rather than on IL-28B levels [Dolganiuc et al., 2012].

In summary, the data reported herein suggested that Allo-SCT recipients harboring the donor IL28B (rs12979860) T/T genotype may expand CMV-specific functional T cells to a higher extent than C/T and C/C patients, which may result in better control of CMV replication early after transplant. Nevertheless, due to the limitations acknowledged above, further studies including larger cohorts are needed to validate our observations.

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REFERENCES

Boeckh M, Ljungman P. 2009. How we treat cytomegalovirus in hematopoietic cell transplant recipients. Blood 113:5711–5719.

Bravo D, Solano C, Giménez E, Remigia MJ, Corrales I, Amat P, Navarro D. 2014. Effect of the IL28B Rs 12979860C/T polymorphism on the incidence and features of active cytomegalovirus infection in allogeneic stem cell transplant patients. J Med Virol 86:838–844.

Chinnaswamy S, Chatterjee S, Boopathi R, Mukherjee S, Bhattacharjee S, Kundu TK. 2013. A single nucleotide polymorphism associated with hepatitis C virus infections located in the distal region of the IL28B promoter influences NF-kappaB-mediated gene transcription. PLoS ONE 8:e75495.

Clari MA, Bravo D, Costa E, Muñoz-Cobo B, Solano C, Remigia MJ, Giménez E, Benmarzouk-Hidalgo OJ, Pérez-Romero P, Navarro D. 2013. Comparison of the new Abbott Real Time CMV assay and the Abbott CMV PCR Kit for the quantitation of plasma cytomegalovirus DNAemia. Diagn Microbiol Infect Dis 75:207–209.

Corrales I, Giménez E, Solano C, Amat P, de la Cámara R, Nieto J, Garcia-Noblejas A, Navarro D. 2015. Incidence and dynamics of active cytomegalovirus infection in allogeneic stem cell transplant patients according to single nucleotide polymorphisms in donor and recipient CCR5, MCP-1, IL-10, and TLR9 genes. J Med Virol 87:248–255.

Dolganiuc A, Kodys K, Marshall C, Saha B, Zhang S, Bala S, Szabo G. 2012. Type III Interferons, IL-28 and IL-29, are increased in chronic HCV infection and induce myeloid dendritic cell-mediated FoxP3+ regulatory T cells. PLoS ONE 7:e44915.

Duggal P, Thio CL, Wojcik GL, Goedert JJ, Mangia A, Latanich R, Kim AY, Lauer GM, Chung RT, Peters MG, Kirk GD, Mehta SH, Cox AL, Khakoo SI, Alric L, Cramp ME, Donfield SM, Edlin BR, Tobler LH, Busch MP, Alexander G, Rosen HR, Gao X, Abdel-Hamid M, Apps R, Carrington M, Thomas DL. 2013. Genome-wide association study of spontaneous resolution of hepatitis C virus infection: Data from multiple cohorts. Ann Intern Med 158:235–245.

Egli A, Silva M, Jr., O'Shea D, Wilson LE, Baluch A, Lisboa LF, Hidalgo LG, Kumar D, Humar A. 2012. An analysis of regulatory T-cell and Th-17 cell dynamics during cytomegalovirus replication in solid organ transplant recipients. PLoS ONE 7: e43937.

Egli A, Levin A, Santer DM, Joyce M, O'Shea D, Thomas BS, Lisboa LF, Barakat K, Bhat R, Fischer KP, Houghton M, Tyrrell DL, Kumar D, Humar A. 2014. Immunomodulatory function of interleukin 28B during primary infection with cytomegalovirus. J Infect Dis 210:717–727.

Fernández-Ruiz M, Corrales I, Arias M, Campistol JM, Giménez E, Crespo J, López-Oliva MO, Beneyto I, Martín-Moreno PL, Llamas-Fuente F, Gutiérrez A, García-Álvarez T, Guerra-Rodríguez R, Calvo N, Fernández-Rodríguez A, Tabernero-Romo JM, Navarro MD, Ramos-Verde A, Aguado JM, Navarro D. 2015. Association between individual and combined SNPs in genes related to innate immunity and incidence of CMV infection in seropositive kidney transplant recipients. Am J Transplant 15:1323–1335.

Ge D, Fellay J, Thompson AJ, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. 2009. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 461:399–401.

Giménez E, Blanco-Lobo P, Muñoz-Cobo B, Solano C, Amat P, Pérez-Romero P, Navarro D. 2015a. Role of cytomegalovirus (CMV)-specific polyfunctional CD8+ T-cells and antibodies neutralizing virus epithelial infection in the control of CMV infection in an allogeneic stem-cell transplantation setting. J Gen Virol 96:2822–2831.

Giménez E, Muñoz-Cobo B, Solano C, Amat P, de la Cámara R, Nieto J, López J, Remigia MJ, Garcia-Noblejas A, Navarro D. 2015b. Functional patterns of cytomegalovirus (CMV) pp65 and immediate early-1-specific CD8(+) T cells that are associated with protection from and control of CMV DNAemia after allogeneic stem cell transplantation. Transpl Infect Dis 17:361–367.

Guerrero A, Riddell SR, Storek J, Stevens-Ayers T, Storer B, Zaia JA, Forman S, Negrin RS, Chauncey T, Bensinger W, Boeckh M. 2012. Cytomegalovirus viral load and virus-specific immune reconstitution after peripheral blood stem cell versus bone marrow transplantation. Biol Blood Marrow Transplant 18:66–75.

Hakki M, Riddell SR, Storek J, Carter RA, Stevens-Ayers T, Sudour P, White K, Corey L, Boeckh M. 2003. Immune reconstitution to cytomegalovirus after allogeneic hematopoietic stem cell transplantation: Impact of host factors, drug therapy, and subclinical reactivation. Blood 102:3060–3067.

Hermant P, Michiels T. 2014. Interferon-λ in the context of viral infections: Production, response and therapeutic implications. J Innate Immun 6:563–574.

Kotenko SV, Gallagher G, Baurin VV, Lewis-Antes A, Shen M, Shah NK, Langer JA, Sheikh F, Dickensheets H, Donnelly RP. 2003. IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. Nat Immunol 4:69–77.

Liu MQ, Zhou DJ, Wang X, Zhou W, Ye L, Li JL, Wang YZ, Ho WZ. 2012. IFN-λ3 inhibits HIV infection of macrophages through the JAK-STAT pathway. PLoS ONE 7:e35902.

Loeffler J, Steffens M, Arlt EM, Toliat MR, Mezger M, Suk A, Wienker TF, Hebart H, Nürnberg P, Boeckh M, Ljungman P, Trenschel R, Einsele H. 2006. Polymorphisms in the genes encoding chemokine receptor 5, interleukin-10, and monocyte chemoattractant protein 1 contribute to cytomegalovirus reactivation and disease after allogeneic stem cell transplantation. J Clin Microbiol 44:1847–1850.

Lu YF, Mauger DM, Goldstein DB, Urban TJ, Weeks KM, Bradrick SS. 2015. IFNL3 mRNA structure is remodeled by a functional non-coding polymorphism associated with hepatitis C virus clearance. Sci Rep 4:16037.

Lui YY, Chik KW, Chiu RW, Ho CY, Lam CW, Lo YM. 2002. Predominant hematopoietic origin of cell-free DNA in plasma and serum after sex- mismatched bone marrow transplantation. Clin Chem 48:421–427.

Manuel O, Wójtowicz A, Bibert S, Mueller NJ, van Delden C, Hirsch HH, Steiger J, Stern M, Egli A, Garzoni C, Binet I, Weisser M, Berger C, Cusini A, Meylan P, Pascual M, Bochud PY. 2015. Influence of IFNL3/4 polymorphisms on the incidence of cytomegalovirus infection after solid-organ transplantation. J Infect Dis 211:906–914.

McFarland AP, Horner SM, Jarret A, Joslyn RC, Bindewald E, Shapiro BA, Delker DA, Hagedorn CH, Carrington M, Gale M Jr., Savan R. 2014. The favorable IFNL3 genotype escapes mRNA decay mediated by AU-rich elements and hepatitis C virus-induced microRNAs. Nat Immunol 15:72–79.

Mezger M, Steffens M, Semmler C, Arlt EM, Zimmer K, Kristjanson GI, Wienker TF, Toliat MR, Kessler T, Einsele H, Loeffler J. 2008. Investigation of promoter variations in dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN) (CD209) and their relevance for human cytomegalovirus reactivation and disease after allogeneic stem- cell transplantation. Clin Microbiol Infect 14:228–234.

Pérez-Romero P, Blanco-Lobo P, Giménez E, Solano C, Navarro D. 2015. An update on the management and prevention of cytomegalovirus infection following allogeneic hematopoietic stem cell transplantation. Future Virol 10:113–134.

Sheppard P, Kindsvogel W, Xu W, Henderson K, Schlutsmeyer S, Whitmore TE, Kuestner R, Garrigues U, Birks C, Roraback J, Ostrander C, Dong D, Shin J, Presnell S, Fox B, Haldeman B, Cooper E, Taft E, Gilbert T, Grant FJ, Tackett M, Krivan W, McKnight G, Clegg C, Foster D, Klucher KM. 2003. IL-28, IL-29 and their class II cytokine receptor IL-28R. Nat Immunol 4:63–68.

Solano C, Muñoz I, Gutiérrez A, Farga A, Prósper F, García-Conde J, Navarro D, Gimeno C. 2001. Qualitative plasma PCR assay (AMPLICOR CMV test) versus pp65 antigenemia assay for monitoring cytomegalovirus viremia and guiding preemptive ganciclovir therapy in allogeneic stem cell transplantation. J Clin Microbiol 39:3938–3941.

Solano C, Benet I, Clari MA, Nieto J, de la Cámara R, López J, Hernández- Boluda JC, Remigia MJ, Jarque I, Calabuig ML, Garcia-Noblejas A, Alberola J, Tamarit A, Gimeno C, Navarro D. 2008. Enumeration of cytomegalovirus-specific interferoná CD8+ and CD4+ T cells early after allogeneic stem cell transplantation may identify patients at risk of active cytomegalovirus infection. Haematologica 93:1434–1436.

Sommereyns C, Paul S, Staeheli P, Michiels T. 2008. IFN-lambda (IFN-lambda) is expressed in a tissue-dependent fashion and primarily acts on epithelial cells in vivo. PLoS Pathog 4: e1000017.

Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, Kidd J, Kidd K, Khakoo SI, Alexander G, Goedert JJ, Kirk GD, Donfield SM, Rosen HR, Tobler LH, Busch MP, McHutchison JG,

Goldstein DB, Carrington M. 2009. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 461:798–801.

Tormo N, Solano C, Benet I, Clari MA, Nieto J, de la Cámara R, López J, López-Aldeguer N, Hernández-Boluda JC, Remigia MJ, Garcia-Noblejas A, Gimeno C, Navarro D. 2010a. Lack of prompt expansion of cytomegalovirus pp65 and IE-1-specific IFNgamma CD8+ and CD4+ T cells is associated with rising levels of pp65 antigenemia and DNAemia during pre-emptive therapy in allogeneic hematopoietic stem cell transplant recipients. Bone Marrow Transplant 45:543–549.

Tormo N, Solano C, Benet I, Nieto J, de la Cámara R, Garcia-Noblejas A, Clari MA, Chilet M, López J, Hernández-Boluda JC, Remigia MJ, Navarro D. 2010b. Kinetics of cytomegalovirus (CMV) pp65 and IE-1-specific IFNgamma CD8+ and CD4+ T cells during episodes of viral DNAemia in allogeneic stem cell transplant recipients: Potential implications for the management of active CMV infection. J Med Virol 82:1208–1215.

Tormo N, Solano C, Benet I, Nieto J, de la Cámara R, López J, Garcia-Noblejas A, Muñoz-Cobo B, Costa E, Clari MA, Hernández-Boluda JC, Remigia MJ, Navarro D. 2011. Reconstitution of CMV pp65 and IE-1-specific IFN-γ CD8(+) and CD4(+) T-cell responses affording protection from CMV DNAemia following allogeneic hematopoietic SCT. Bone Marrow Transplant 46:1437–1443.

Yoshio S, Kanto T, Kuroda S, Matsubara T, Higashitani K, Kakita N, Ishida H, Hiramatsu N, Nagano H, Sugiyama M, Murata K, Fukuhara T, Matsuura Y, Hayashi N, Mizokami M, Takehara T. 2013. Human blood dendritic cell antigen 3 (BDCA3)(+) dendritic cells are a potent producer of interferon-λ in response to hepatitis C virus. Hepatology 57:1705–1715.

Zhang S, Kodys K, Li K, Szabo G. 2013. Human type 2 myeloid dendritic cells produce interferon-lambda and amplify interferon-alpha in response to hepatitis C virus infection. Gastroenterology 144:414–425.e7.

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