Immunomodulatory Function of Interleukin 28B During Primary Infection With Cytomegalovirus

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Background. Feedback mechanisms between interferons α and λ (IFNs) may be affected by single nucleotide polymorphisms (SNP) in interleukin 28B (IL-28B; IFN-λ3) promoter region and may influence cytomegalovirus (CMV) replication.

Methods. We associated IL-28B SNPs with the risk of CMV replication after transplantation. Next, we examined the effect of IL-28B genotypes on IL-28B, and IFN-stimulated gene (ISG) expression, and CMV replication in human foreskin fibroblast (HFF) and peripheral blood mononuclear cells (PBMCs).

Results. Transplant recipients with an IL-28B SNP (rs8099917) had significantly less CMV replication ($P = .036$). Both HFF-cells and PBMCs with a SNP showed lower IL-28B expression during infection with CMV, but higher "antiviral" ISG expression (eg, OAS1). Fibroblasts with a SNP had a 3-log reduction of CMV replication at day 4 ($P = .004$). IL-28B pretreatment induced ISG expression in noninfected fibroblasts, but a relative decrease of ISG expression could be observed in CMV-infected fibroblasts. The inhibitory effects of IL-28B could be abolished by siRNA or antagonistic peptides against the IL-28 receptor. In fibroblasts, inhibition of IL-28 signaling resulted in an increase of ISG expression and 3-log reduction of CMV-replication ($P = .01$).

Conclusions. We postulate that IL-28B may act as a key regulator of ISG expression during primary CMV infection. IL-28B SNPs may be associated with higher antiviral ISG expression, which results in better replication control.

Keywords. cytomegalovirus; interferon λ; interleukin 28; solid organ transplantation; immunosuppression; T-cell priming; innate immune response; adaptive immune response; feedback mechanism; interferon-stimulated gene.

The interferon (IFN)-λ family comprises: interleukin (IL)-28A, -28B, and -29 and IFN-λ4 [1, 2]. All have been ascribed antiviral properties similar to IFN-α [3, 4]. In vivo, IFN-λs are mainly secreted by dendritic cells (DC) and macrophages [2, 5]. Evidence for a potential interaction between IFN-λ and IFN-α was revealed in genome-wide association studies (GWAS) of patients with chronic HCV infection. Single nucleotide polymorphisms (SNP) in the IL-28B promoter are associated with lower rates of HCV clearance in response to IFN-α treatment [6–9]. These SNPs are divided according to the frequencies in the population: for the rs8099917 SNP, TT and for the rs12979860 SNP, CC represent the most frequently observed genotypes in whites and Asians ("major" alleles); for rs8099917 SNPs, TG and GG and for rs12979860 SNPs, CT and TT are termed "minor" allele genotypes [6–9]. These SNPs potentially impact the binding of transcription factors and methylation sites [10–13]. Some of these SNPs may be co-linked with other functional SNPs. Liver biopsies in patients with chronic HCV infection indicated that the IL-28B genotype alters IL-28B expression [14, 15], as well as in peripheral blood

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mononuclear cells (PBMCs) [16]. Minor allele genotypes have been associated with increased ISG expression, which may thus inhibit viral replication [17–20]. The role of IL-28B SNPs in acute viral infection outside of HCV is not understood.

In immunocompromised hosts, such as transplant recipients, acute CMV infection is associated with high morbidity [21, 22]. Immunosuppression contributes significantly to the loss of CMV-specific adaptive immune control [23]. In this context the role of innate immunity to control CMV replication is magnified. CMV has evolved many ways to modulate the innate and adaptive immune response [24]; in particular, modulation of the IFN-α and its associated pathway have been described [25]. Whether IFN-λ contributes to innate responses, in the context of acute CMV infection is not known. Transplant recipients who are CMV-seronegative and receive an allograft from a CMV-seropositive donor (D + R−) are at highest risk of CMV-associated morbidity. These patients routinely undergo antiviral prophylaxis [22]. However, after prophylaxis discontinuation they also present a unique opportunity to study the course of primary CMV infection [26].

We aimed to explore the effect of IL-28B SNPs on acute CMV infection and innate immune signalling. In addition, we examined the impact of IL-28B on CMV-specific T-cell priming. Detailed knowledge of these control mechanisms in the context of primary CMV infection have implications for the clinical practice of CMV-disease prevention, utilization of antiviral therapy in immunosuppressed patients, as well as the development of a CMV vaccine.

MATERIALS AND METHODS

Patient Population

Solid organ transplant patients at high risk for CMV replication (D + R−) were enrolled at the University of Alberta. CMV viremia was monitored after discontinuation of prophylaxis by quantitative polymerase chain reaction (PCR), measuring plasma CMV DNA level [27] (Supplementary Figure 1*A*). Healthy volunteers were recruited for T-cell priming experiments. The study protocols were approved through local ethics committees and written informed consent was provided.

IL-28B Promoter Region Polymorphism Genotyping

SNP genotypes were determined using Taqman probes on a 7900HT Fast Real-Time PCR system (Life Technologies). Probes for the rs12979860 SNP (ABI Assay ID AHS0QIE) were published elsewhere [28]. This probe set discriminates the C and T alleles, where C is the major and T is the minor allele. Probes for the rs8099917 SNP were designed using the Taqman assay design tool (ABI Assay ID AH1RU99). At this locus, T predicts the major allele, whereas G predicts the minor allele. Samples including positive controls were run in duplicate.

Cells and Cell Cultures

Human foreskin fibroblasts (HFF) from ATCC (HS97FS, SCRC1041, and CCD1112SK, Supplementary Table 1*A*) and peripheral blood mononuclear cells (PBMCs) were grown in standard media. PBMCs were isolated as published elsewhere [29].

Cytomegalovirus and Plaque Assay

We used the laboratory adapted Towne strain of CMV for all assays. To determine viral growth we infected HFF cells and treated them as described in the individual experiments. Cells and supernatant were harvested postinfection. Then, we used standard 14-day plaque assays to determine viral growth.

IL-28A, IL-28B, and IL-29 Specific Primers and Probes

IL-28B primers/probe were designed based on human interleukin-28B (IFN-λ3), IL-28A (IFN-λ2), and IL-29 (IFN-λ1) messenger RNA (mRNA) sequences using Primer3 Input (version 0.4.0; http://frodo.wi.mit.edu/). Supplementary Table 1*B* shows all primers and probes (Integrated DNA technologies). Specificity was tested against expression plasmids containing complete IFNλ1-3 (Invitrogen, puno1-hil28a/28b/29) [2] (data not shown).

mRNA-expression Profile: IFN-α/β/λ, Pro- and Anti-inflammatory ISGs

RNA was extracted and after reverse transcription, mRNA expression was quantified using a real-time PCR approach using 10 ng of complementary DNA (cDNA) per reaction. Expression profiles were normalized to controls (media) and hypoxanthine phosphoribosyltransferase (HPRT) using the Pfaffl method [30]. The following primers and probes were used (TaqMan gene expression assays): Interferon alpha-2 (IFNα2, Hs00265051_s1), Interferon beta-1 (IFNβ1, Hs01077958_s1), MX1 (Hs00895608_m1), OAS1 (Hs00973637_m1), IFIT2 (Hs 00533665_m1), ISG15 (Hs01921425_s1), SOCS1 (00705164_ s1), USP18 (Hs00276441_m1), HPRT (Hs01003267_m1), and IL-28RA (Hs00417120_m1). In the case of no signal at 40 cycles in unstimulated samples, a CT value of 40 was set as detection for relative mRNA expression.

ELISA IFN-α

IFN-α levels in supernatants were quantified by an in-house enzyme-linked immunosorbent assay (ELISA) using commercially available antibodies and a human IFN-α standard (PBL Biomedical Laboratories). The detection limit was 4.7 pg/mL.

siRNA Transfection

Small interfering RNA (siRNA) against IL-28B and IL-28RA was used at a concentration of 25 nM (Mission siRNA, EHu065311-20 μg; negative control, mission siRNA SIC001-10 μMol; IL-28B SRSI-hs01-00171730, all Sigma). Lipofectamin

(RNAiMax, Invitrogen) was used for transfection according to the manufacturer.

Western Blots

Proteins were harvested using RIPA lysis buffer (Santa Cruz Biotechnology). Samples were loaded at 10 µg of protein/lane in standard loading buffer to a discontinuous (4.0%/7.5%) Laemmli/SDS-PAGE gel. Standard running conditions and wet transfer were used. Primary antibodies against IL-28B and IL-28RA (Abcam), and against STAT2, and phosphorylated-STAT2, and GAPDH (RnD), followed by secondary HRP-conjugated antibodies (Santa Cruz Biotechnology).

Design of IL28 Antagonistic Peptides

Based on previous publications [31, 32] of the crystal structures of IL-29 and the receptor IL-28RA (PDB: 3OG4, 3OG6), we determined the amino acid residues, which are in close proximity to mediate interaction between the 2 molecules. We used the crystal structure of IL-28B oligomer (PDB: 3HHC [31]) focusing on amino acids, whose residues may be involved in the interactions responsible for the formation of the oligomeric state. We then designed peptides (Supplementary Table 1*C*) to mimic these interaction domains in order to prevent the formation of oligomers.

Flow Cytometry

Prior to surface staining, LIVE/DEAD staining was performed (near-IR; invitrogen). Markers for identifying T-cell subsets were CD3, CD4, CD8, CD45RA, and CD69. Monocytes were labelled with CD14, MHC-II, CD40, and CD86 (all antibodies from Biolegend or eBiosciences). For T-cell expansion experiments, PBMCs were labelled with Cell Trace Violet proliferation dye (Invitrogen). Stimulation was according to the respective experimental condition for 5 days in 5%CO_2 at 37°C.

Statistical Analysis

Statistical analyses were performed using SPSS (version 18.0, Chicago) and GraphPad Prism (version 4.0, La Jolla). Categorical variables were evaluated using a χ^2 test. Data were nonnormally distributed according to Shapiro–Wilk testing; therefore, continuous variables between 2 groups were evaluated with a Mann–Whitney *U* test. A 2-tailed *P*-value of <.05 was considered significant.

RESULTS

Transplant Recipients (D + R−) With Major-allele Genotype Show More CMV Replication Within the First Year Post Transplant

We recruited 38 D + R− patients at high risk for CMV primary infection. Table 1 shows details of patient characteristics. Genotypic distribution is comparable to that of the general population (data not shown; [6–9]). The duration of antiviral prophylaxis, allograft types, immunosuppression, patient age, and gender were comparable across groups. In accordance with guidelines all D+/R− patients received antiviral prophylaxis [22]. Interestingly, after stopping antiviral prophylaxis, CMV replication was detected significantly more often in transplant recipients with a major-allele genotype in rs8099917 (TT vs TG/GG 45% vs 22% $P = .036$; OR 5.62 95% CI, 1.02–31.10; Table 2). Figure 1*B* illustrates a Kaplan–Meier analysis comparing patients with major (TT) and minor allele genotypes (TG or GG). For the rs12979860 SNP a trend was observed for more CMV replication in the major allele genotype (CC vs TT, 52% vs 0%; $P = .089$; Table 3). In addition, we analyzed the data without the multivisceral and heart transplant recipients. Although the power of the study was reduced, a strong statistical trend remained for the rs8099917 ($P = .1$) and rs12979860 ($P = .09$) SNPs.

Table 1. Baseline Characteristics and Genotype Distribution

Characteristic	Measure or Criteria	N = 38
Age, years	Median (IQR)	47.5 (22)
Gender	Male	29 (76.3%)
	Female	9 (23.7%)
Type of graft	Kidney	14 (36.8%)
	Liver	7 (18.4%)
	Heart	2 (5.3%)
	Lung	9 (23.7%)
	Small bowel	1 (2.6%)
	Kidney and pancreas	4 (10.5%)
	Multivisceral	1 (2.6%)
CMV replication		17 (44.7%)

Age, gender, type of transplant, and rate of CMV replication. Age in years indicates the age at the time of enrolment into the study. CMV replication indicates active CMV replication within 12 months of antiviral prophylaxis discontinuation.

Abbreviations: CMV, cytomegalovirus; IQR, interquartile range.

Human Foreskin Fibroblasts With Major-allele Genotype Show Higher CMV Replication and Lower CMV-induced ISG Expression

Next, we used HFF cells as a model system for CMV replication. HFF cells harboring different IL-28B genotypes were used to

Table 2. Effects of rs8099917 on Cytomegalovirus (CMV) Replication

	CMV Replication			
rs8099917	No	Yes	Total	χ^2
TT	12 (55%)	15 (45%)	27	
TG or GG	9 (77.8%)	2 (22.2%)	11	0.036
	21	17	38	

Transplant recipients with a minor allele (GT or GG) genotype experienced less CMV replication.

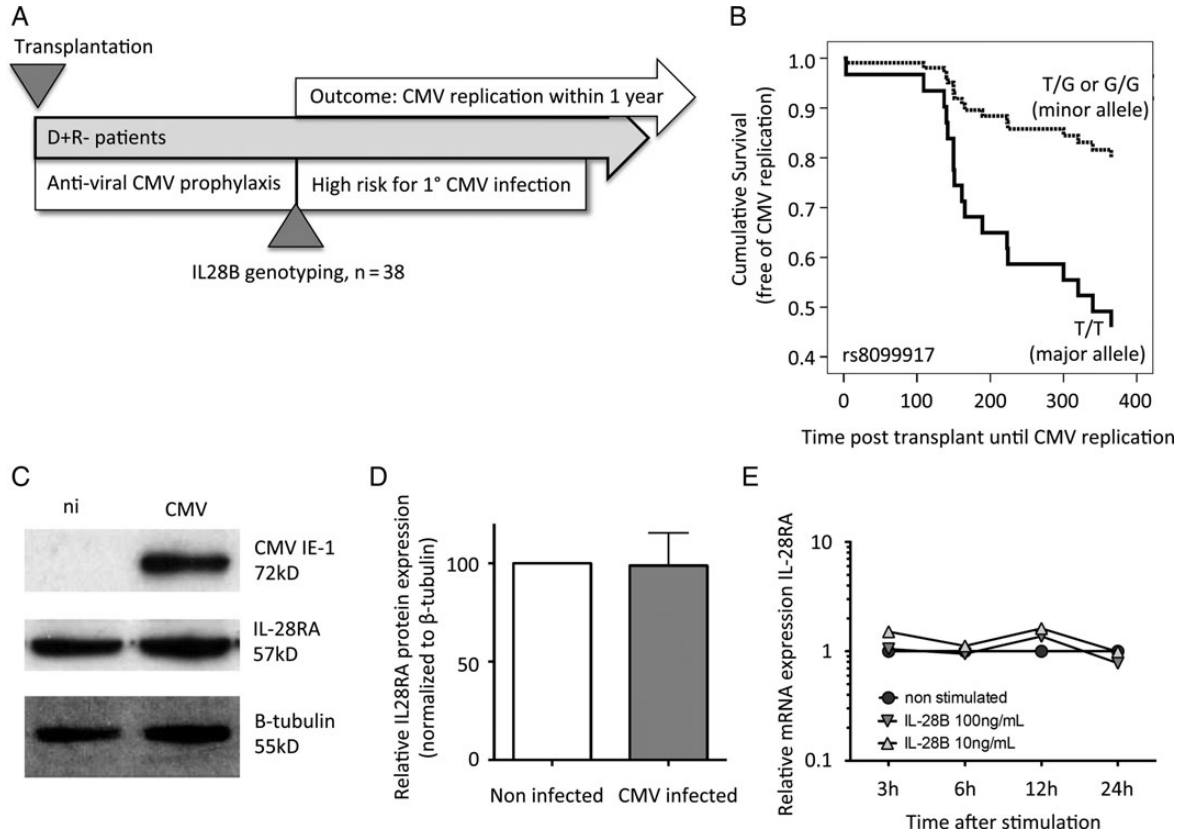

Figure 1. *A*, Study design: CMV replication after stop of antiviral prophylaxis. Patients (n = 38) at high risk for CMV primary infection were prospectively recruited. After discontinuation of standard Ganciclovir/Valganciclovir prophylaxis, CMV replication was monitored using a preemptive strategy during a 1-year follow-up period. *B*, CMV replication after transplantation according to IL-28B genotype. Cox regression indicates that patients carrying a minor allele in rs12979860 (TG or GG; *dotted line*) show less primary CMV-replication after transplantation compared to patients carrying a major-allele (TT, *solid line*). *C–E*, Protein and mRNA expression of IL-28RA during CMV-infection. *C*, Western blot of HFF-cells with IL-28RA, CMV- immediate early 1(IE1) and beta-Tubulin. HFF cells were infected (CMV Towne, MOI 0.3) for 2 hours and compared to noninfected (ni) cells, washed and incubated for 48 hours prior to receptor expression measurement. Western blot of a representative experiments is shown. *D*, Relative IL-28RA mRNA expression normalized to beta-Tubulin is shown. Eight independent experiments were performed. No significant differences in IL-28RA expression were observed between CMV infection and noninfection. *E*, mRNA expression profile of IL-28RA in HFF during stimulation with polyI:C. HFF cells were pretreated with recombinant IL-28B (10 ng/mL and 100 ng/mL) before stimulation with polyI:C (50 μg/mL). mRNA expression is normalized against HPRT and non-IL-28B treated expression. No significant modulation of IL-28RA upon different IL-28B dosages was observed. Abbreviations: CMV, cytomegalovirus; HFF, human foreskin fibroblast; HPRT, hypoxanthine phosphoribosyltransferase; IL, interleukin; mRNA, messenger RNA.

investigate the dynamics of CMV infection in vitro (Supplementary Table 1*A*). We focused on the manifestations of the IL-28B SNP rs12979860 (CT or TT), because HFF cells carrying rs8099917 SNPs (TG or GG) were not available via ATCC, and we could not recruit HFF cells from newborns in our center. IL-28 receptor α-subunit (IL-28RA) expression was constant during CMV infection (Figure 1*C–E*). CMV growth curves indicated that the major-allele genotype was associated with 3- log10 higher CMV replication at day 4 when compared with the minor allele ($P = .004$, Figure 2*A*).

To further characterize the effect of the rs12979860 SNP, we measured IFN-λ and IFN-α2 mRNA expression in HFF cells. HFF cells with a major-allele genotype (CC) had higher IL-28B mRNA expression during CMV infection compared to minor-allele genotypes (CT or TT) (Figure 2*B*). In contrast to IL-28B, IFNα2 mRNA- expression was lower during CMV infection in HFF cells with the major-allele genotype (CC) compared to minor-allele genotype (CT or TT). Although IFN-α2 mRNA-expression peaked 6 hours after CMV infection, the difference between genotypes was maximal at 12 hours and 24 hours (Figure 2*C*).

IFN-α2 and ISG expression are associated. Therefore, we examined the expression of 2 sets of antiviral ISGs: MX1, OAS1, IFIT2, and ISG15 were compared to anti-inflammatory ISGs: USP18 and SOCS1 in each of the HFF cells during CMV infection. Interestingly, the cell line with the major allele genotype

Table 3. Effects of the rs12979860 on Cytomegalovirus (CMV) Replication

	CMV Replication			
rs12979860	No	Yes	Total	χ^2
CC	10 (48%)	11 (52%)	21	
TT	3 (100%)	0 (0%)	3	0.089
	13	11	24	

Transplant recipients with a minor-allele genotype (TT) showed a trend to less CMV replication.

(CC) had lower levels of antiviral ISGs (Figure 2*D*–*E*) and higher levels of anti-inflammatory ISGs (Figure 2*F*). In HFF cells with the major allele, IFIT2 expression was induced approximately 6-fold less by CMV infection; conversely, USP18 was induced 3-fold more.

Interferon-λ Pretreatment of HFF Cells Results in Lower IFN-α2 Signaling and Pro-inflammatory ISG Induction in Response to CMV Infection

Because we observed a correlation between IL-28B genotype and antiviral response in HFF cells, we determined the effects of exogenously added recombinant IFN-λs. The purpose of this was to mimic a strong "major allele"-like situation. We observed a dose-dependent inhibitory effect of IFN-λs pretreatment on CMV-induced IFN-α2 mRNA expression in HFF cells (SCRC1041, CT genotype; Figure 3*A*). In addition, we observed a strong suppressive effect on antiviral ISG expression during CMV infection by IL-28B (Figure 3*B*). In contrast, anti-inflammatory ISGs (USP18 and SOCS1), remained upregulated at later time-points of infection in HFF cells pretreated with IL-28B (Figure 3*C*). Supplementary Figure 1*A* illustrates the induction of ISGs based solely on IFN-λ treatment in non-infected HFF cells (Supplementary Figure 1*A*). These findings highlight that IFN-λ induces ISGs, but in the context of CMV

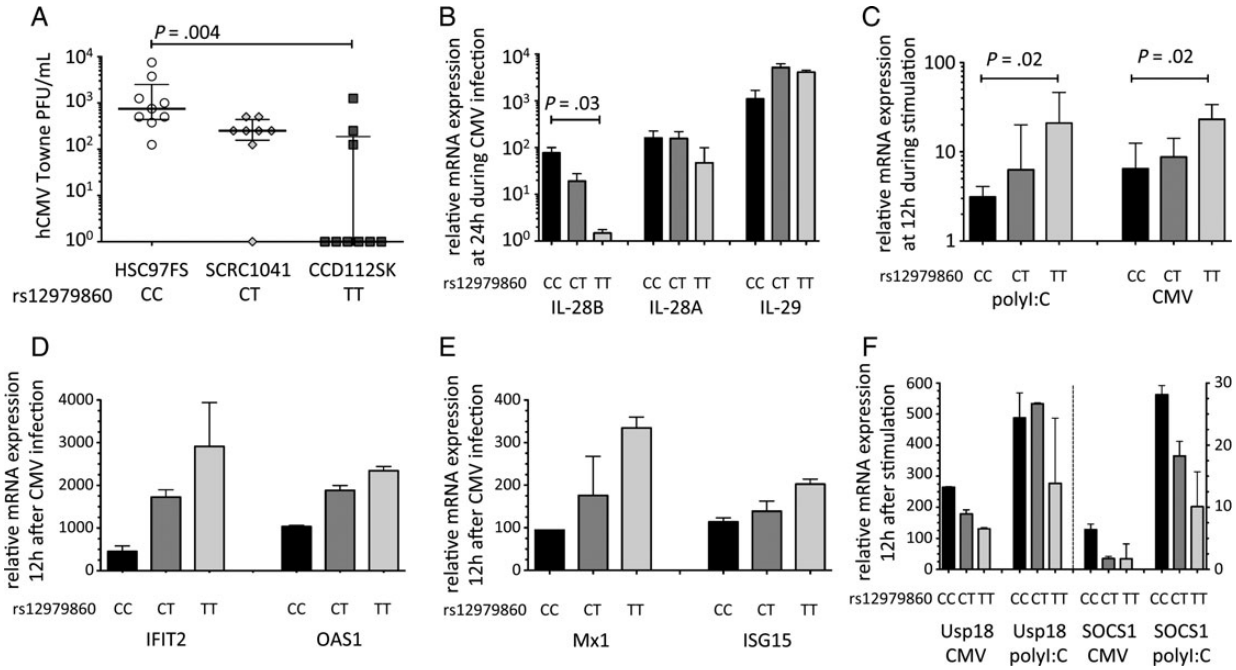

Figure 2. IL-28B SNP in fibroblasts reduces CMV replication by increased CMV-induced IFN-α and reduced IFN-λ responses. HFF cells with different IL-28B genotypes (HS97FS rs12979860: CC, rs8099917: TT; SCRC1041 rs12979860: CT, rs8099917: TT; and CCD1112SK rs12979860: TT, rs8099917: TT) were infected with human CMV (Towne strain, MOI 0.3). mRNA expression is relative to HPRT and non-infected controls, and 3 independent experiments were performed unless otherwise indicated. Bars or symbols indicate median value, whiskers the interquartile ranges unless otherwise indicated. The rs8099917 was the same for all tested cell lines (major allele genotype). *A*, CMV replication in HFF cells measured with plaque assays. Supernatants were collected at day 4 from 9 individual experiments. Viral growth was determined using plaque assays as described, and viral load is expressed as plaque-forming units (PFU) per mL of supernatant. *B*, IFN-λ mRNA-expression of infected fibroblasts. IL-28A, IL-28B, and IL-29 mRNA is expressed at 24 hours. *C*, IFN-α2 mRNA expression of stimulated or infected fibroblasts. In addition to infection (as previously described), a stimulation with poly I:C (50 μg/mL) was performed. mRNA expression of IFN-α2 is shown at 12 hours. *D*, Proinflammatory ISG response (IFIT2 and OAS1) during CMV infection of HFF cells. *E*, Proinflammatory ISG response (Mx1 an ISG15) during CMV infection of HFF cells. *F*, Anti-inflammatory ISG response (SOCS1 and USP18) during infection and stimulation of HFF cells. Same infection condition as previously describe were used, but in this experiment stimulation was performed with transfected poly I:C (7.5 μg/mL). Abbreviations: CMV, cytomegalovirus; HFF, human foreskin fibroblast; IFN, interferon; IL, interleukin; ISG, IFN-stimulated gene; mRNA, messenger RNA.

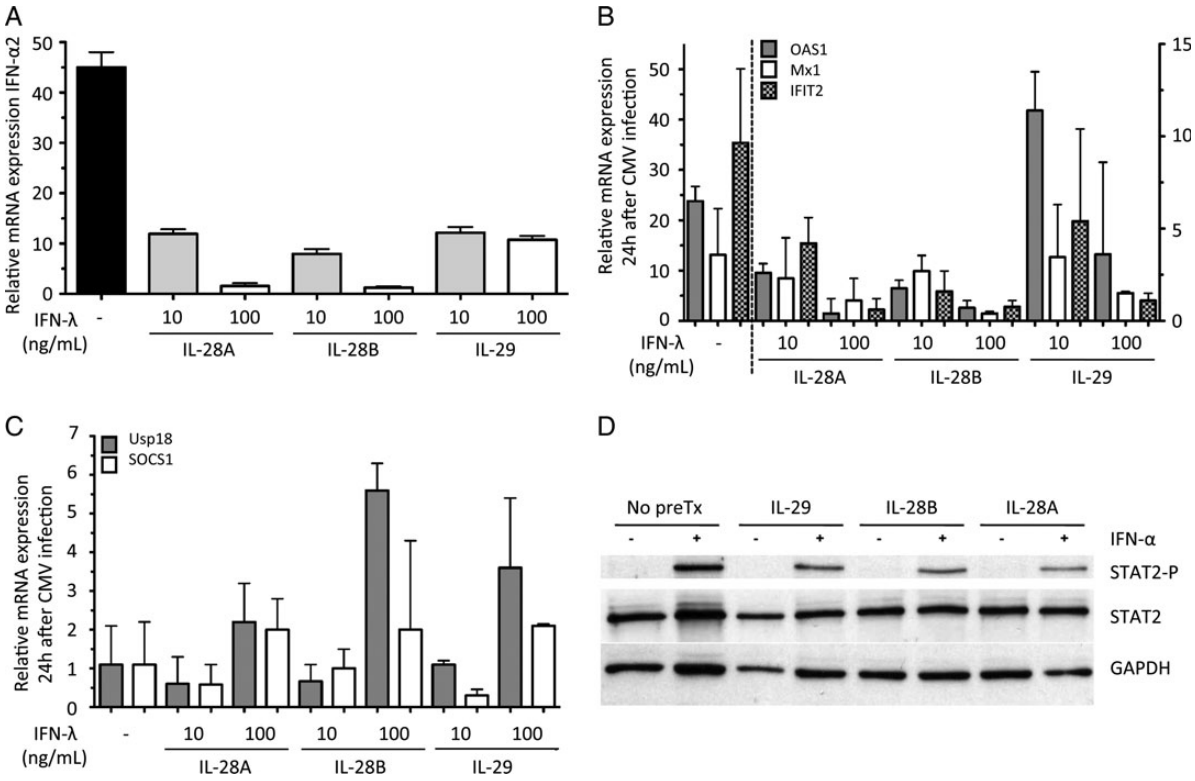

Figure 3. Effects of IFN-λ on IFN-α induced signaling in fibroblasts. mRNA expression is relative to HPRT and noninfected controls, and 3 independent experiments were performed unless otherwise indicated. Bars or symbols indicate median value, whiskers the interquartile ranges unless otherwise indicated. IFN-λ (100 ng/mL) was added 2 hours prior to infection with CMV (MOI 0.3). Data from 3 independent experiments are shown. *A*, Effects of IFN-λs on IFNα-response during infection of HFF-cells. mRNA expression is shown 6 hours after infection. *B*, Effect of IFN-λ on pro-inflammatory ISG expression during CMV infection. mRNA-expression profile is shown 24 hours after infection. *C*, Effect of IFN-λ on anti-inflammatory ISG expression during CMV infection. mRNA expression is shown 24 hours after infection. *D*, Effects of IFN-λ on STAT-phosphorylation during IFNα-receptor activation. HFF cells were pretreated with IFN-λ for 8 hours, then washed and rested for another 16 hours. Finally, IFN-α2 (10 IU/mL) was added to the cells for 30 minutes prior to harvesting. Abbreviations: CMV, cytomegalovirus; HFF, human foreskin fibroblast; IFN, interferon; IL, interleukin; ISG, IFN-stimulated gene; mRNA, messenger RNA.

infection IL-28B effects extensive modulation of net ISG expression.

Because SOCS-1 inhibits signaling via the IFN-α/β receptor [33, 34], we determined the effect of IFN-λs on STAT2-phosphorylation. Pretreatment of HFF cells with recombinant IFN-λs for 8 hours, followed by a resting phase of 16 hours, and then 30 minutes IFN-α2 treatment. IL-28B reduced IFN-α2 induced STAT-2 phosphorylation by 60% (MWU $P = .06$; Figure 3*D*, Supplementary Figure 1*B*).

Inhibition of Interferon-λ Signaling Increases Pro-inflammatory ISG Expression and Reduces CMV Replication

Since pre-incubation of HFF cells with IFN-λ resulted in decreased IFN-α2 signaling and decreased pro-inflammatory ISG expression in response to CMV infection, we determined whether inhibition of IFN-λ signaling could result in the converse situation—increased ISG expression and lower CMV replication. These experiments were performed in cells with a CT genotype background (SCRC1041). Transfection of siRNA against IL-28RA resulted in reduction of IL-28RA expression in HFF cells compared to control siRNA (Supplementary Figure 1*C* and 1*D*). Down-regulation of IL-28RA had a significant effect on CMV replication compared to negative control siRNA ($P = .007$). We observed a decline in CMV replication by a median of greater than a 2-log10 at day-4 post-infection (Figure 4*A*). This effect was not caused by toxicity due to transfection of siRNA (MTT-assay, data not shown). Consistent with our previous experiments, inhibition of IFN-λ signaling using siRNA resulted in a greater induction of pro-inflammatory ISGs during CMV infection (Figure 4*B*).

In addition, we designed peptides with the purpose of modulating IL-28A/B and IL-29 induced signaling via the inhibition of interactions with IL-28RA (Figure 4*C*–*H*). The peptides were screened based on their ability to inhibit IL-28B signaling. Peptides PRT, LKY, and LNC (10 μM) were added to HFF cells for 2 hours, followed by a 30 minutes challenge with IL-28B (100 μg/mL). Signaling was assessed by STAT2-phosphorylation through Western blot. Peptides LKY and LNC lead to a 40% and 34% reduction in STAT2-phosphorylation, respectively.

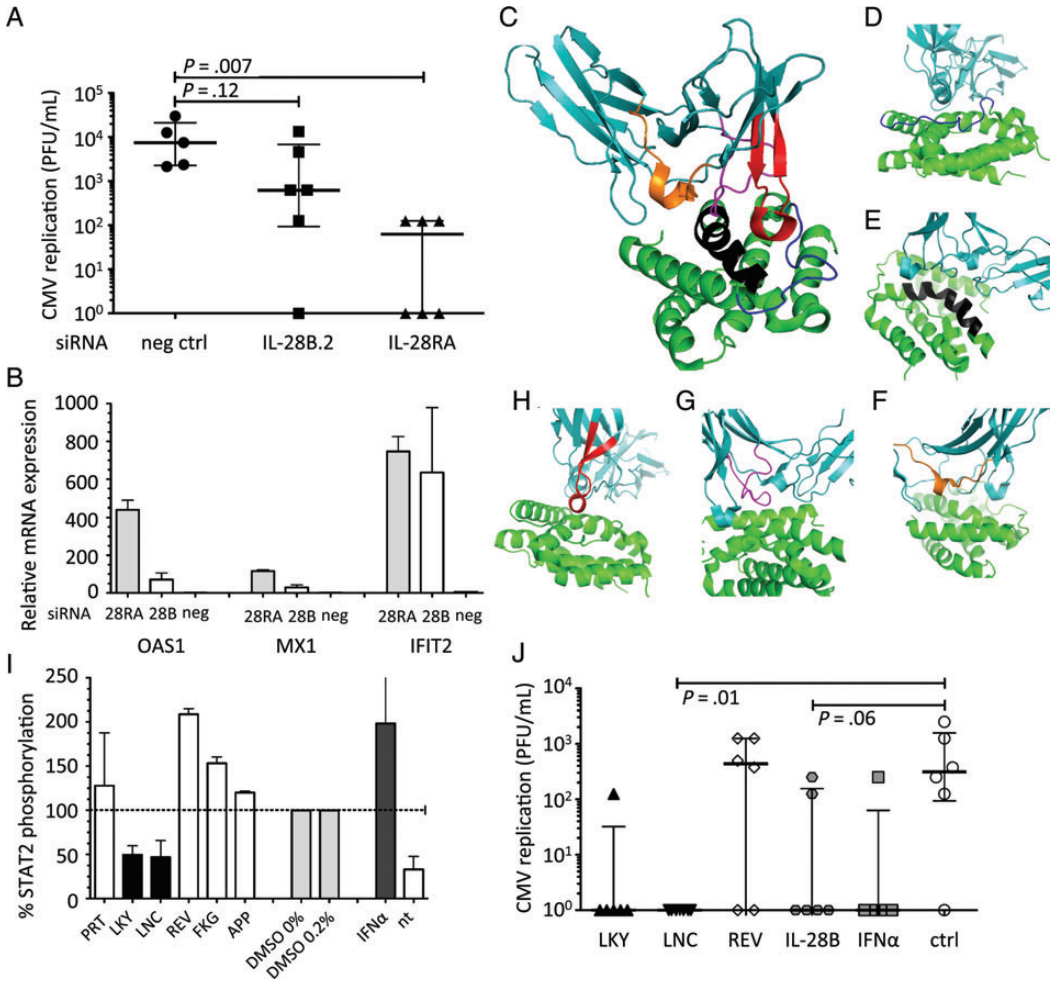

Figure 4. Stimulatory effects of IFN-λ signaling inhibition. siRNA transfection against IL-28RA and IL28B (25 nM) and respective control siRNA (25 nM) for 72 hours prior to infection with CMV (Towne, MOI 0.03). Bars show median values, whiskers indicate interquartile ranges. mRNA expression is normalized to HPRT and non-antigen control siRNA. *A*, Effects of siRNA against IL-28RA and IL-28B on the replication of CMV in fibroblasts. Harvest of cell culture supernatants at day 4. Determination of viral growth using plaque assays. *B*, Effects of siRNA induced suppression of IL-28B and its receptor IL-28RA subunit on pro-inflammatory ISGs. Down-regulation of IL-28B and its receptor was associated with an increase in pro-inflammatory ISGs expression compared to negative control siRNA. *C–H*, Design of peptides with potential to interfere with the IL-28RA pathway. Interaction of the IL-29 natural ligand with IL-28RA is shown. In color different fragments serving as backbone for the blocking peptides is shown. *I*, Blocking potential of peptides. Peptides PRT, LKY, and LNC (10 μM) were preincubated for 2 hours with HFF cells, washed, and were challenged with IL-28B (100 ng/mL) for 30 minutes. Peptides REV, FKG, APP were preincubated for 2 hours with IL-28B and added to HFF1 for 30 minutes. Proteins were harvested and STAT2 phosphorylation was determined relative to STAT2 and β-tubulin and expressed as percent of respective DMSO controls. Data of 3 independent experiments. Peptide LKY, LNC, and APP were dissolved in 0.2% DMSO, whereas the other peptides were dissolved in water (a representative Western blot is shown in Supplementary Figure 1*E*). Peptides were excluded from further characterization if they independently activated STAT2-phosphorylation (data not shown) *J*, Effects of blocking peptides on CMV replication in fibroblasts. HFF cells were pretreated for 2 hours with peptides (10 μM) and respective DMSO control (ctrl). CMV replication was determined at day 4 using plaque assays. Abbreviations: CMV, cytomegalovirus; DMSO, dimethyl sulfoxide; HFF, human foreskin fibroblast; IFN, interferon; IL, interleukin; ISG, IFN-stimulated gene; mRNA, messenger RNA; siRNA, small interfering RNA.

Peptides REV, FKG, and APP (10 μM) were pre-incubated with recombinant IL-28B (100 ng/mL) for 2 hours before being added to HFF cells for 30 minutes. None of these peptides blocked STAT-2 phosphorylation (Figure 4*I*, Supplementary Figure 1*E*). Consistent with the ability to inhibit signaling by IL-28B, pretreatment of HFF cells with peptide LNC resulted in 2.5 log10 lower CMV-replication at day 4 after infection (MWU, $P = .01$; Figure 4*J*).

The IL-28B SNP and Recombinant IL-28B Modulate IFN Responses in PBMCs and Inhibit T-cell Priming During CMV-infection

Successful control of virus-replication is induced and maintained by monocytes, T- and B-cells. In order to further examine the effect of IL-28B, PBMCs from healthy donors were in vitro stimulated with CMV. IL-28B mRNA expression was 2000-fold higher in PBMCs from rs8099917 and/or

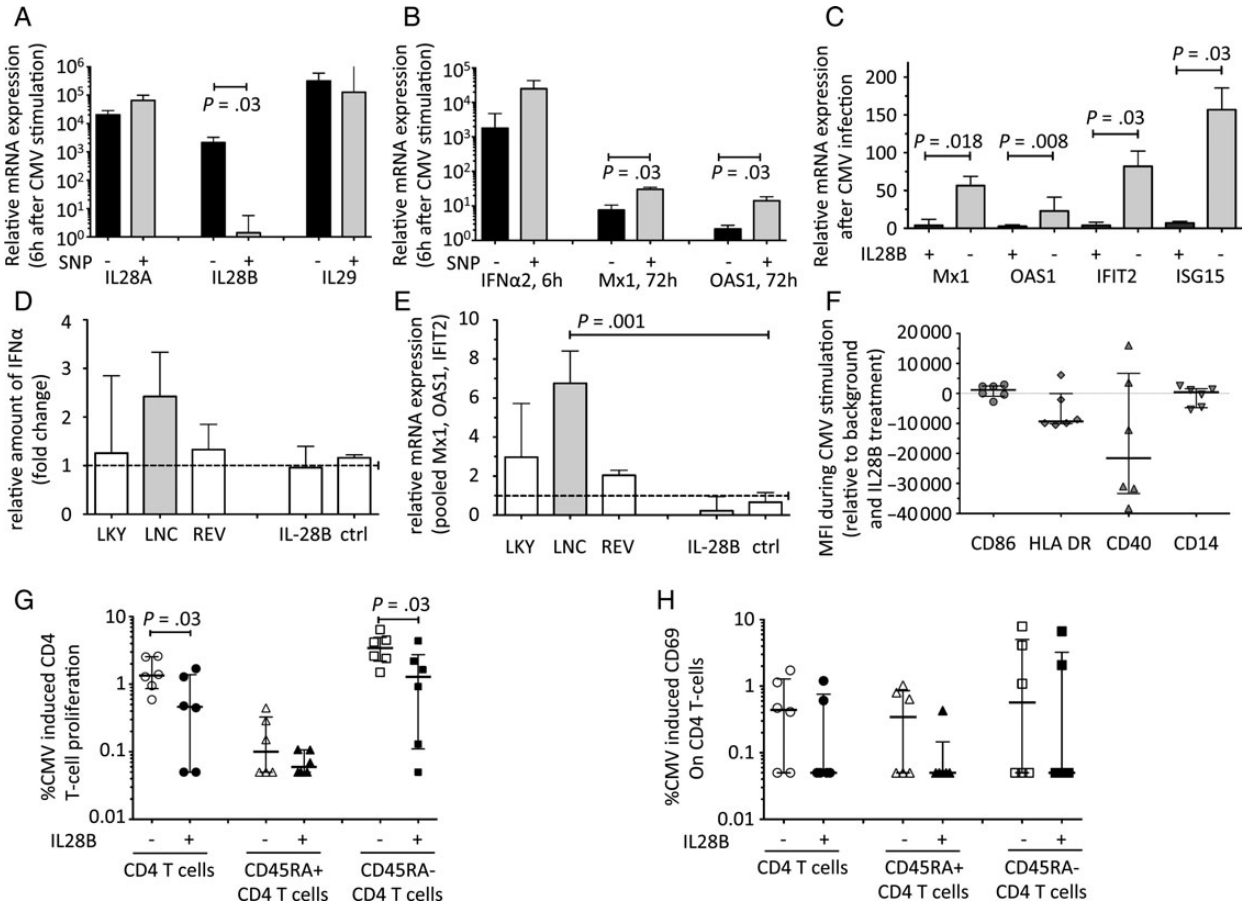

Figure 5. Modulating effects of IL-28B and IL-28B SNPs on interferon responses and on adaptive immune functions during T-cell priming. PBMCs from healthy volunteers were stimulated with CMV (MOI 0.3). IFN-λ and ISG mRNA-expressions was determined *A* and *B*, mRNA was normalized to HPRT expression and relative fold increases were calculated to non-stimulated controls. Bars indicate median values, whiskers interquartile range values. Comparison between 4 major-allele carriers and 4 minor-allele carriers are shown. *A*, CMV induced IFN-λ mRNA expression according to IL-28B genotype. mRNA-expression is shown 6 hours after stimulation. *B*, Stimulatory effect of CMV on pro-inflammatory ISG mRNA expression according to genotype. IFNα2 mRNA-expression is shown 6 hours later, and MX1 and OAS1 mRNA expression are shown at 72 hours time point. *C*, Effect of IL-28B on pro-inflammatory ISG expression. PBMCs were pretreated for 2 hours with recombinant IL-28B (100 ng/mL) and then stimulated with CMV (MOI 0.3). MX1, OAS1, IFIT2, ISG15 mRNA-expression is shown at 72 hours irrespective of the IL-28B genotype. Comparisons between 7 individuals are shown. *D*, Effects of pretreatment of PBMC with peptides on CMV stimulated IFN-α production. Irrespective of the IL28B genotype, PBMC from healthy volunteers (n = 5) were pretreated with peptides (10 μM) for 2 hours prior to stimulation with CMV (MOI 0.3). IFNα levels were determined by ELISA at 24 hours. IFNα is shown as fold change compared to non-peptide treated controls. *E*, Effects of pre-treatment of PBMC with peptides on CMV stimulated ISG mRNA expression. Relative ISG expressions of MX1, OAS1, and IFIT2 were pooled in a profile for every single treatment condition (a separation is shown in Supplementary Figure 2*A*). *F*, Impact of IL-28B on antigen presentation and co-stimulatory signaling of monocytes. PBMCs from CMV seronegative healthy blood donors were pretreated with recombinant IL-28B (100 ng/mL) prior to a 5-day stimulation with CMV (MOI 0.05). Relative MFI expression of CD86, HLA DR, CD40 and CD14 is shown normalized to noninfected and non-pretreated fibroblast lysate (raw data of the experiment is provided in Supplementary Figure 2*B*). *G*, Impact of IL-28B on priming of naive T cells. PBMCs from CMV seronegative healthy volunteers were pretreated with recombinant IL-28B (100 ng/mL) prior to a 5-day stimulation with CMV (MOI 0.3). T-cell proliferation during in vitro priming with CMV is expressed in percent of the overall respective CD4 T-cell population. *H*, Impact of IL-28B on T-cell activation after expansion phase. T cells of different subtypes show a clear down-regulation of CD69 surface expression. Abbreviations: CMV, cytomegalovirus; ELISA, enzyme-linked immunosorbent assay; HFF, human foreskin fibroblast; IFN, interferon; IL, interleukin; ISG, IFN-stimulated gene; mRNA, messenger RNA; PBMC, peripheral blood mononuclear cells; siRNA, small interfering RNA.

rs12979860 major-allele carriers following stimulation with CMV compared to minor-allele carriers (Figure 5*A*).

PBMCs from donors with a minor-allele genotype exhibited a 10-fold higher mRNA-expression of IFN-α2 following CMV stimulation compared to minor-allele genotypes (Figure 5*B*). IFN-α expression was not accounted for by differences in plasmacytoid dendritic cells, gender, and age (data not shown). As observed in HFF-cell lines, major-allele carriers displayed lower antiviral ISG expression during CMV stimulation compared with minor-allele carriers (Figure 5*B*). Pretreatment with IL-28B (100 ng/mL) for 2 hours prior to stimulation with CMV suppressed antiviral ISG expression at later time points,

providing more evidence that this effect is IL-28B dependent (Figure 5*C*, Supplementary Figure 1*F*).

In order to further explore the modulatory impact of IL-28B, we used our antagonistic peptides to pretreat PBMCs for 2 hours prior to 24 hours CMV stimulation. Peptide LNC resulted in a 2.4-fold induction of IFN-α2 production measured by ELISA. In addition, the same peptide induced a panel of antiviral ISGs (MX1, OAS1, and IFIT2) by more than 6-fold compared to respective negative controls (MWU, $P = .001$) (Figure 5*D*–*E*). Supplementary Figure 2*A* shows mRNA expression changes for individual ISGs (Supplementary Figure 2*A*).

IL-28B Impairs Priming of the Adaptive Immune Response to CMV

Given that IFN-α2 is an important cofactor in activating antigen-presenting cells and promoting antigen presentation to T cells [35], we determined the impact of IL-28B on monocyte activation, and priming of T cells in PBMCs of CMV-seronegative healthy nonimmunsuppressed blood donors. Pretreatment with recombinant IL-28B prior to CMV stimulation showed a clear trend to reduce CD40 and HLA-DR expression on monocytes (CD14+, Figure 5*F*, Supplementary Figure 2*B*) and B cells (CD20+, data not shown). CD40 is a crucial co-stimulatory factor for T cells and provides activation signals for monocytes [36] and B cells [37].

IL-28B was associated with a significant reduction of naive T-cell priming during in vitro CMV stimulation ($P = .03$, Figure 5*G*). The inhibitory effect during the priming phase was reflected by a significant reduction of naive CD4+ CD45RA- T-cell proliferation (>50%) during stimulation with CMV over 5 days. In addition, this important T-cell subset, which later configures the memory compartment, had a notable down-regulation of CD69 (median >30%), an important T-cell activation marker (Figure 5*H*).

DISCUSSION

This study provides novel insight into the interplay between IFN-α and IFN-λ in the context of CMV infection. We demonstrate that IL-28B may act as a key regulator of ISG expression and plays a role in T-cell priming during acute CMV infection. Transplant recipients at high-risk for primary CMV infection, who carry a minor-allele genotype, showed less CMV replication during the first year following transplantation. Employing detailed in-vitro and ex-vivo analysis we propose that IL-28B itself, and the IL-28B SNP mediate this clinical observation via a modulation of IFN signalling, induced interferon-stimulated genes, and T-cell priming (Figure 5).

Recently, in hematopoietic stem cell transplant recipients with a high risk for CMV primary infection, the IL-28B minor allele genotype was associated with a lower risk of infection as well as shorter episode of CMV replication [38]. These data highly support our hypothesis. In contrast, the IL-28B minor-allele genotype is known to negatively influence HCV treatment outcomes [6–9]. Interestingly, several studies observed an elevated ISG expression in chronic HCV minor-allele carriers [17–19, 39–41]—giving rise to 2 potential interpretations. First, HCV is exposed to a generally higher baseline ISG expression favouring selection of resistant quasi-species [20]. Second, a higher set-point of ISG impedes a significant induction with IFN-α treatment.

Interestingly, in liver transplant patients with a minor-allele phenotype in graft and recipient the initial viral RNA level at HCV recurrence is significantly lower, suggesting a phase of superior antiviral control [42, 43]. The somewhat surprising association of higher ISG expression in patients with a minor-allele genotype suggests a feedback between IFN-λ and IFN-α.

Host cells induce IFN-α, IFN -β, and IFN-λ during CMV infection [44, 45]. However, the complex role of IFN-λ in the context of CMV replication and its interplay with other IFNs is incompletely characterized. Our in vitro experiments show that in fibroblasts, the minor-allele genotype was associated with reduced CMV replication. Fibroblasts with a minor-allele genotype showed significantly lower IL-28B mRNA expression during CMV infection. Interestingly, this was associated with higher IFN-α and antiviral ISG expression. We demonstrated that treatment with IL-28B reversed these effects and suppressed IFN-α and antiviral ISGs during CMV infection. The induction of anti-inflammatory ISGs likely inhibits STAT2-phosphorylation [33, 46]. A recent study confirmed that priming of hepatocytes with IL-29 impaired IFN-α induced STAT phosphorylation via USP18 [46]. In hepatocytes stimulated with IFN-λ and IFN-α, an overlap of ISG induction was observed; however, striking differences were present in the array and duration of induced genes [47, 48]. Interestingly, IFN-λ induced anti-inflammatory genes, such as SOCS1, to a much greater extent than IFN-α [47].

The IL-28 receptor (IL-28RA) transmits the regulatory effects of IFN-λ. Therefore, we were interested in the potential of IL-28RA blockade. We suppressed the IFN-λ signalling by reducing the ligand-receptor interaction using siRNA knockdown of the IL-28RA. This resulted in significantly increased ISG expression and reduced CMV replication. One limitation is that transfection with siRNA in itself may change IFN expression, even though each experiment was performed with a concurrent negative control siRNA. Also, we designed peptides able to bind and inhibit the IL-28RA. Pretreatment of HFF-cells with blocking peptides resulted in increased antiviral ISGs and reduced CMV replication.

Aside from the important functions of the innate immune system, the adaptive immune response is also crucial in efficiently controlling virus replication. CMV-specific naive T cells have an important role in the control of primary CMV infection [23]. We provide strong evidence that the adaptive

immune response during primary CMV infection might be modulated by IFN-λ. In particular, naive T cells are inhibited in their proliferative potential and activation state. Interestingly, blockade of the "IL-28-receptor" markedly reduced the RSV-mediated suppression of CD4 T-cell proliferation [49]. Although T cells do not express IL-28RA receptor on the cell surface, the effect of IL-28B may be transmitted via monocytes, which show significant IL-28RA expression (data not shown). Our data suggest that IL-28B regulates HLA-DR and CD40 expression on monocytes in the context of CMV-stimulation. Both antigen presentation and co-stimulatory signalling to naive T cells are critical elements in the priming phase.

A limitation of this study is the relatively low number and heterogeneity of patients included. However, this unique patient group of transplant recipients at highest risks for primary CMV infection presents the rare opportunity to study the influence of innate immune variations. The clinical implication of these findings will have to be confirmed in other cohorts. We excluded seropositive solid organ transplant recipients as the immunological processes to CMV reactivation are different, and therefore these patients may show a different outcome. CMV has DNA proof-reading, therefore the accumulation of ISG resistance mutations is less frequent compared to RNA viruses (see discussed above). In a R+ patient, latent infection is already established, and the control of virus replication in this situation has different requirements [23].

Although, post-translational modifications of ISG-mRNA could occur, we demonstrate that IFN-λ inhibits the down-stream signalling of IFN-α. Additionally, the blockade of IL-28RA signalling by differing means and the use of a plaque assay to measure CMV replication provides robust data regarding the importance of these pathways. IFN-α or IFN-λ specific ISGs should be identified using microarray expression profiles and might help to further dissect the down-stream effects. Another limitation is the cell types and CMV strains used in this study: further evaluation could be performed using different strains of CMV (including clinical isolates) and different cell lines such as endothelial cells. In addition, performing in-vitro experiments using cells from the transplant recipients may provide additional supportive data. Finally, we were unsuccessful in obtain an HFF-cell line with the rs8099917 SNP, and it would be important for future studies to further analyse the in-vitro effects of this SNP, perhaps by screening other sources of fibroblasts such as newborn foreskins, in order to obtain one with this SNP.

In summary, we have demonstrated that immunosuppressed patients carrying the minor-allele IL-28B SNP have superior control of CMV replication following primary infection. IL-28B specifically inhibited IFN-α-induced ISG expression and hindered the priming of CMV-specific T cells. These findings stand to have a considerable impact upon the clinical management of CMV screening and prophylaxis in immunosuppressed patients. Finally, manipulation of the IFN-λ pathway can prove valuable in terms of augmenting adaptive immune responses, such as those required for effective vaccination.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Booth D, George J. Loss of function of the new interferon IFN-lambda4 may confer protection from hepatitis C. Nat Genet **2013**; 45:119–20.
2. Kotenko SV, Gallagher G, Baurin VV, et al. IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. Nat Immunol **2003**; 4:69–77.
3. Kelly C, Klenerman P, Barnes E. Interferon lambdas: the next cytokine storm. Gut **2011**; 60:1284–93.
4. Sadler AJ, Williams BR. Interferon-inducible antiviral effectors. Nat Rev Immunol **2008**; 8:559–68.
5. Yin Z, Dai J, Deng J, et al. Type III IFNs Are Produced by and Stimulate Human Plasmacytoid Dendritic Cells. J Immunol **2012**; 189:2735–45.
6. Rauch A, Kutalik Z, Descombes P, et al. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology **2010**; 138:1338–45, 45 e1–7.
7. Tanaka Y, Nishida N, Sugiyama M, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet **2009**; 41:1105–9.
8. Suppiah V, Moldovan M, Ahlenstiel G, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet **2009**; 41:1100–4.
9. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature **2009**; 461:399–401.
10. Bibert S, Roger T, Calandra T, et al. IL28B expression depends on a novel TT/-G polymorphism which improves HCV clearance prediction. J Exp Med **2013**; 210:1109–16.

11. Chinnaswamy S, Chatterjee S, Boopathi R, Mukherjee S, Bhattacharjee SKundu TK. 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 **2013**; 8: e75495.
12. Fischer J, Bohm S, Scholz M, et al. Combined effects of different interleukin-28B gene variants on the outcome of dual combination therapy in chronic hepatitis C virus type 1 infection. Hepatology **2012**; 55:1700–10.
13. Smith KR, Suppiah V, O'Connor K, et al. Identification of improved IL28B SNPs and haplotypes for prediction of drug response in treatment of hepatitis C using massively parallel sequencing in a cross-sectional European cohort. Genome Med **2011**; 3:57.
14. Sugiyama M, Tanaka Y, Wakita T, Nakanishi M, Mizokami M. Genetic variation of the IL-28B promoter affecting gene expression. PLoS One **2011**; 6:e26620.
15. Dill MT, Duong FH, Vogt JE, et al. Interferon-induced gene expression is a stronger predictor of treatment response than IL28B genotype in patients with hepatitis C. Gastroenterology **2011**; 140:1021–31.
16. Shi X, Pan Y, Wang M, et al. IL28B genetic variation is associated with spontaneous clearance of hepatitis C virus, treatment response, serum IL-28B levels in Chinese population. PLoS One **2012**; 7:e37054.
17. Abe H, Hayes CN, Ochi H, et al. Inverse association of IL28B genotype and liver mRNA expression of genes promoting or suppressing antiviral state. J Med Virol **2011**; 83:1597–607.
18. Sarasin-Filipowicz M, Krol J, Markiewicz I, Heim MH, Filipowicz W. Decreased levels of microRNA miR-122 in individuals with hepatitis C responding poorly to interferon therapy. Nat Med **2009**; 15:31–3.
19. Honda M, Sakai A, Yamashita T, et al. Hepatic ISG expression is associated with genetic variation in interleukin 28B and the outcome of IFN therapy for chronic hepatitis C. Gastroenterology **2010**; 139:499–509.
20. Maekawa S, Sakamoto M, Miura M, et al. Comprehensive analysis for viral elements and il28b polymorphisms in response to peginterferon plus ribavirin therapy in HCV-1b infection. Hepatology **2012**; 56:1611–21.
21. Gandhi MK, Khanna R. Human cytomegalovirus: clinical aspects, immune regulation, and emerging treatments. Lancet Infect Dis **2004**; 4:725–38.
22. Humar A, Snydman D. Cytomegalovirus in solid organ transplant recipients. Am J Transplant **2009**; 9 (Suppl 4):S78–86.
23. Egli A, Humar A, Kumar D. State-of-the-art on monitoring of cytomegalovirus-specific cell mediated immunity after organ transplant - a primer for the clinician. Clin Infect Dis **2012**; 55:1678–89.
24. Ploegh HL. Viral strategies of immune evasion. Science **1998**; 280:248–53.
25. Loewendorf A, Benedict CA. Modulation of host innate and adaptive immune defenses by cytomegalovirus: timing is everything. J Intern Med **2010**; 267:483–501.
26. Humar A, Mazzulli T, Moussa G, et al. Clinical utility of cytomegalovirus (CMV) serology testing in high-risk CMV D+/R- transplant recipients. Am J Transplant **2005**; 5:1065–70.
27. Lisboa LF, Asberg A, Kumar D, et al. The clinical utility of whole blood versus plasma cytomegalovirus viral load assays for monitoring therapeutic response. Transplantation **2011**; 91:231–6.
28. Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature **2009**; 461:798–801.
29. Egli A, Binet I, Binggeli S, et al. Cytomegalovirus-specific T-cell responses and viral replication in kidney transplant recipients. J Transl Med **2008**; 6:29.
30. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res **2001**; 29:e45.
31. Gad HH, Dellgren C, Hamming OJ, Vends S, Paludan SR, Hartmann R. Interferon-lambda is functionally an interferon but structurally related to the interleukin-10 family. J Biol Chem **2009**; 284:20869–75.
32. Miknis ZJ, Magracheva E, Li W, Zdanov A, Kotenko SV, Wlodawer A. Crystal structure of human interferon-lambda1 in complex with its high-affinity receptor interferon-lambdaR1. J Mol Biol **2010**; 404: 650–64.
33. Fenner JE, Starr R, Cornish AL, et al. Suppressor of cytokine signaling 1 regulates the immune response to infection by a unique inhibition of type I interferon activity. Nat Immunol **2006**; 7:33–9.
34. Waiboci LW, Ahmed CM, Mujtaba MG, et al. Both the suppressor of cytokine signaling 1 (SOCS-1) kinase inhibitory region and SOCS-1 mimetic bind to JAK2 autophosphorylation site: implications for the development of a SOCS-1 antagonist. J Immunol **2007**; 178: 5058–68.
35. Spadaro F, Lapenta C, Donati S, et al. IFN-alpha enhances cross-presentation in human dendritic cells by modulating antigen survival, endocytic routing, and processing. Blood **2012**; 119:1407–17.
36. Alderson MR, Armitage RJ, Tough TW, Strockbine L, Fanslow WC, Spriggs MK. CD40 expression by human monocytes: regulation by cytokines and activation of monocytes by the ligand for CD40. J Exp Med **1993**; 178:669–74.
37. Fecteau JF, Neron S. CD40 stimulation of human peripheral B lymphocytes: distinct response from naive and memory cells. J Immunol **2003**; 171:4621–9.
38. Bravo D, Solano C, Gimenez E, et al. Effect of the IL28B Rs12979860 C/T polymorphism on the incidence and features of active cytomegalovirus infection in allogeneic stem cell transplant patients. J Med Virol **2014**; 86:838–44.
39. Chen L, Borozan I, Feld J, et al. Hepatic gene expression discriminates responders and nonresponders in treatment of chronic hepatitis C viral infection. Gastroenterology **2005**; 128:1437–44.
40. Feld JJ, Nanda S, Huang Y, et al. Hepatic gene expression during treatment with peginterferon and ribavirin: identifying molecular pathways for treatment response. Hepatology **2007**; 46:1548–63.
41. Lau DT, Negash A, Chen J, et al. Innate immune tolerance and the role of Kupffer cells in differential responses to interferon therapy among patients with HCV genotype 1 infection. Gastroenterology **2013**; 144:402–13.
42. Duarte-Rojo A, Veldt BJ, Goldstein DD, et al. The course of posttransplant hepatitis C infection: comparative impact of donor and recipient source of the favorable IL28B genotype and other variables. Transplantation **2012**; 27:197–203.
43. Cisneros E, Banos I, Citores MJ, et al. Increased risk of severe hepatitis C virus recurrence after liver transplantation in patients with a T allele of IL28B rs12979860. Transplantation **2012**; 94:275–80.
44. Paludan SR, Bowie AG, Horan KA, Fitzgerald KA. Recognition of herpesviruses by the innate immune system. Nat Rev Immunol **2011**; 11:143–54.
45. Brand S, Beigel F, Olszak T, et al. IL-28A and IL-29 mediate antiproliferative and antiviral signals in intestinal epithelial cells and murine CMV infection increases colonic IL-28A expression. Am J Physiol Gastrointest Liver Physiol **2005**; 289:G960–8.
46. Francois-Newton V, Magno de Freitas Almeida G, Payelle-Brogard B, et al. USP18-based negative feedback control is induced by type I and type III interferons and specifically inactivates interferon alpha response. PLoS One **2011**; 6:e22200.
47. Thomas E, Gonzalez VD, Li Q, et al. HCV infection induces a unique hepatic innate immune response associated with robust production of type III interferons. Gastroenterology **2012**; 142:978–88.
48. Marcello T, Grakoui A, Barba-Spaeth G, et al. Interferons alpha and lambda inhibit hepatitis C virus replication with distinct signal transduction and gene regulation kinetics. Gastroenterology **2006**; 131:1887–98.
49. Chi B, Dickensheets HL, Spann KM, et al. Alpha and lambda interferon together mediate suppression of CD4 T cells induced by respiratory syncytial virus. J Virol **2006**; 80:5032–40.