Immunoactivation induced by chronic viral infection inhibits viral replication and drives immunosuppression through sustained IFN-I responses

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Acute or chronic viral infections can lead to generalized immunosuppression. Several mechanisms, such as immunopathology of CD8^{+} T cells, inhibitory receptors, or regulatory T (Treg) cells, contribute to immune dysfunction. Moreover, patients with chronic viral infections usually do not respond to vaccination, a finding that has not been previously explained. Recently, we reported that CD169^{+} macrophages enforce viral replication, which is essential for guaranteeing antigen synthesis and efficient adaptive immune responses. In the present study, we used a chronic lymphocytic choriomeningitis virus infection mouse model to determine whether this mechanism is affected by chronic viral infection, which may impair the activation of adaptive immunity. We found that enforced viral replication of a superinfecting virus is completely blunted in chronically infected mice. This absence of enforced viral replication in CD169^{+} macrophages is not explained by CD8^{+} T-cell-mediated immunopathology but rather by prolonged IFN-I responses. Consequently, the absence of viral replication impairs both antigen production and the adaptive immune response against the superinfecting virus. These findings indicate that chronic infection leads to sustained IFN-I action, which is responsible for the absence of an antiviral immune response against a secondary viral infection.

Keywords: CD169^{+} macrophages · Chronic infection · Enforced virus replication · Lymphocytic choriomeningitis virus · Vesicular stomatitis virus

☒ See accompanying Commentary by John R. Teijaro

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Introduction

Many acute and chronic viral infections induce a generalized immunosuppression [1, 2] in a broad range of host species [3, 4]. During acute infection, immunosuppression is usually transient but can last for several months after the initial replication has been controlled. In contrast, persistent infection with certain viruses can lead to lifelong immunosuppression [5].

In humans, acute infection with the measles virus can cause a generalized immunosuppression lasting for several months and can facilitate bacterial superinfection [6–8]. Persistent infections with a low viral load, such as CMV and EBV infections, are known to negatively influence the immune response against other viruses (i.e. influenza virus) [9, 10]. An increase in the number of regulatory T cells in mice chronically infected with Friend retrovirus suppresses immunity to a superinfection with murine CMV [11].

Infection with HIV or with human T-cell lymphotropic virus leads to severe immunosuppression and a poor response against infection with several opportunistic pathogens [1, 12]. This finding is explained primarily by the infection of CD4+ T cells. Additionally, several studies using the lymphocytic choriomeningitis virus (LCMV) model have shown that NK cells, the development or reduction in the number of dendritic cells (DCs), and CD8+ T-cell-mediated immunopathology contribute to immunosuppression [2, 13–15]. This finding is in line with observations from studies of infections with other viruses showing that constant activation of CD8+ T cells in lymph nodes and spleen alters immune function [2, 11]. It has been reported that LCMV infects B cells that produce neutralizing antibodies, which will be eliminated by immunopathological LCMV-specific CD8+ T cells [2]. This LCMV infection also induces a highly specific immunosuppression that delays the responses of neutralizing antibody and thereby enhances viral persistence [5, 13, 16]. A comparison of various strains of LCMV found that a higher rate of viral replication is associated with the severity and duration of immunosuppression. For example, infection with the LCMV strain Docile, which causes chronic infection, leads to a strong suppression of the immune system, whereas infection with the LCMV strain Armstrong causes only slight immunosuppression [5]. Although the immunosuppression is explained by the immunopathology related to CD8+ T cells, it still remains unclear why mice infected with LCMV-Docile exhibit immunosuppression despite the exhaustion of CD8+ T cells.

Recent studies have shown that chronic infection with LCMV Clone 13 leads to an increase in the production of type I IFN, which in turn can increase the production of the immunosuppressive cytokine IL-10 [17, 18]. Studies using monkeys such as sooty mangabeys and rhesus macaques found that the onset of acquired immunodeficiency syndrome (AIDS) is strongly correlated with levels of type I IFN [19]. The mechanism by which type I IFN induces immunosuppression remains unknown.

Recently we reported that special APCs, such as metallophilic marginal zone macrophages (CD169+), in the spleen and DCs express *Usp18*, which regulates host cell susceptibility to type I

IFN and supports viral replication [20, 21]. This enforced viral replication is essential for initiating innate and adaptive immune responses. Whether the mechanism of enforced viral replication is influenced by chronic viral infection remains unknown.

Results

Chronic infection limits a secondary antiviral immune response

To determine the effect of a chronic infection on the suppression of the adaptive immune system, we infected C57BL/6 mice with LCMV strain Docile 30 days before superinfection with the vesicular stomatitis virus (VSV). We infected another group of C57BL/6 mice with VSV alone to serve as controls (Fig. 1A). We measured the production of VSV-neutralizing antibodies in the serum at various time points. We found that chronic infection with LCMV Docile blunted the induction of total neutralizing antibodies (IgM and IgG) and of neutralizing IgG against VSV (Fig. 1B). This shows that chronic infection can lead to suppression of the activity of the adaptive immune system against a secondary viral infection.

Enforced viral replication is blunted in chronically infected mice

In a previous study we found that enforced viral replication is essential for activating adaptive immunity and producing neutralizing antibodies against VSV [20]. We questioned whether chronic infection may influence the mechanism of enforced viral replication. To study this hypothesis, we infected mice with LCMV-Docile for 30 days. Mice were then superinfected with VSV for 7 h, and the viral titer in spleen and inguinal lymph nodes was determined. Control mice were infected with VSV alone. VSV replication was lower in mice infected with LCMV-Docile than in control mice (Fig. 2A).

The reduction of VSV replication by chronic LCMV infection was additionally assessed by immunohistological staining of spleen sections (Fig. 2B). To determine whether this immunosuppression was due to the inhibition of enforced viral replication, we used VSV that had been inactivated with UV light to immunized naive mice and mice with chronic LCMV infection. Both groups of mice exhibited similar titers of total neutralizing and IgG-neutralizing antibodies (Fig. 2C), a finding suggesting that B cells can still produce antibodies during chronic infection. These findings suggest that the persistence of LCMV infection could inhibit enforced viral replication of the superinfected virus.

CD8+ T cells do not prevent immunosuppression during chronic infection

Next, we questioned whether this suppression of the B-cell response was due to CD8+ T-cell-mediated cytotoxicity during
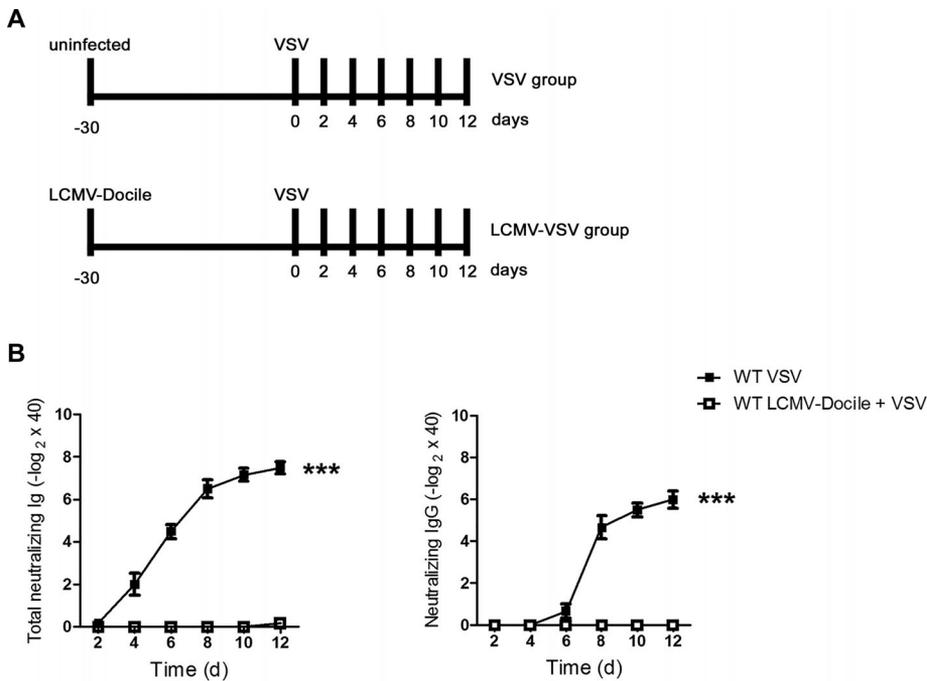
A

B

Figure 1. Chronic infection limits antiviral immune response. (A) Experimental protocol shows infection time points. (B) C57BL/6 WT mice were chronically infected with 2×10^4 PFU LCMV strain Docile (LCMV-Docile) for 30 days or left uninfected. VSV-neutralizing antibodies were measured in chronically LCMV-infected or uninfected WT mice intravenously infected with 1×10^4 PFU VSV at the indicated time points by neutralizing assay. Data are expressed as mean ± SEM ($n = 4$–6 mice/group) and are pooled from two independent experiments. ***$p <$ 0.001. Statistical significance was detected with ANOVA.

chronic LCMV infection. To answer this question, we infected perforin-deficient mice ($Prf^{-/-}$) with LCMV-Docile, and 30 days later we superinfected them with VSV. A control group of $Prf^{-/-}$ mice was infected with VSV alone. Perforin is the primary cytotoxic effector protein in CD8$^+$ T cells [22]. We found that $Prf^{-/-}$ mice infected with chronic LCMV and then superinfected with VSV exhibit a much more impaired B-cell response than do $Prf^{-/-}$ mice infected with VSV alone (Fig. 3A). Superinfected $Prf^{-/-}$ mice also exhibited an inhibition in VSV replication similar to that of coinfected WT mice (Figs. 2B and 3B). These findings exclude a cytotoxic role of CD8$^+$ T cells in immunosuppression and suggest that another perforin-independent mechanism leads to immunosuppression during chronic LCMV infection. In order to completely exclude the role of CD8$^+$ T cells and not only perforin-mediated cytotoxic effect, we infected MHC-I-deficient mice with LCMV-Docile, and after 30 days we superinfected them with VSV. As control, we infected MHC-I-deficient mice only with VSV. Similar to $Prf^{-/-}$ mice, deletion of MHC-I did not prevent the immunosuppression induced by chronic LCMV-Docile infection (Fig. 3C). Additionally, superinfected $MHC-I^{-/-}$ mice showed an inhibition in VSV replication similar to superinfected WT mice (Figs. 2B and 3D). We conclude that CD8$^+$ T cells are not involved in the immunosuppression.

Persistent viral infection can also occur if newborn mice are infected with virus. In this case, central tolerance is induced against the virus, and virus-specific T cells are deleted [23]. Therefore, we questioned whether even with the deletion of LCMV-specific T cells, the immunosuppression and blunting of viral replication during neonatal infection is taking place. We therefore infected neonatal mice with LCMV-Docile and then superinfected them with VSV. As controls, another group of C57BL/6 mice was infected with VSV alone (Fig. 3E). After superinfection with

VSV, VSV replication was blunted in mice infected with LCMV as neonates (Fig. 3F). In addition, mice infected as neonates exhibited a slight reduction in the titers of total neutralizing antibody (IgM and IgG) and a massive reduction in the titers of neutralizing IgG against VSV (Fig. 3G). These findings led us to conclude that enforced viral replication is also blunted in mice infected as neonates, a conclusion that can explain the reduction in the adaptive immune response after secondary viral infection.

Prolonged activity of type I IFN inhibits enforced viral replication during chronic infection

Chronic LCMV infection leads to prolonged type I IFN responses [18, 24]. We hypothesized that enhanced type I IFN responses would then induce an antiviral status via *Oas1* and *Mx1*, which render APCs resistant to viral replication. We measured gene expression in spleens stimulated with type I IFN during chronic LCMV infection and compared it with gene expression under naïve conditions. Indeed, we found that the expression of *Stat1*, *Irf7*, *Oas1*, and *Mx1* was higher during chronic infection (Fig. 4A). Moreover, chronically infected mice exhibited stronger expression of *Ifnα4* and *Ifnβ1* than did uninfected mice (Fig. 4A). To determine whether mice infected with LCMV as neonates also exhibit stronger type I IFN signaling and higher type I IFN expression, we measured the expression of these genes. As expected, mice infected as neonates exhibit sustained type I IFN activity (Fig. 4B).

To determine whether this prolonged type I IFN activity can inhibit the enforced viral replication of VSV, we infected WT mice with LCMV-Docile and after 30 days we treated them with anti-IFN-αR1 or isotype control antibody. Afterward, we superinfected them with VSV. Immunohistological staining of spleen sections showed higher replication of VSV in superinfected WT
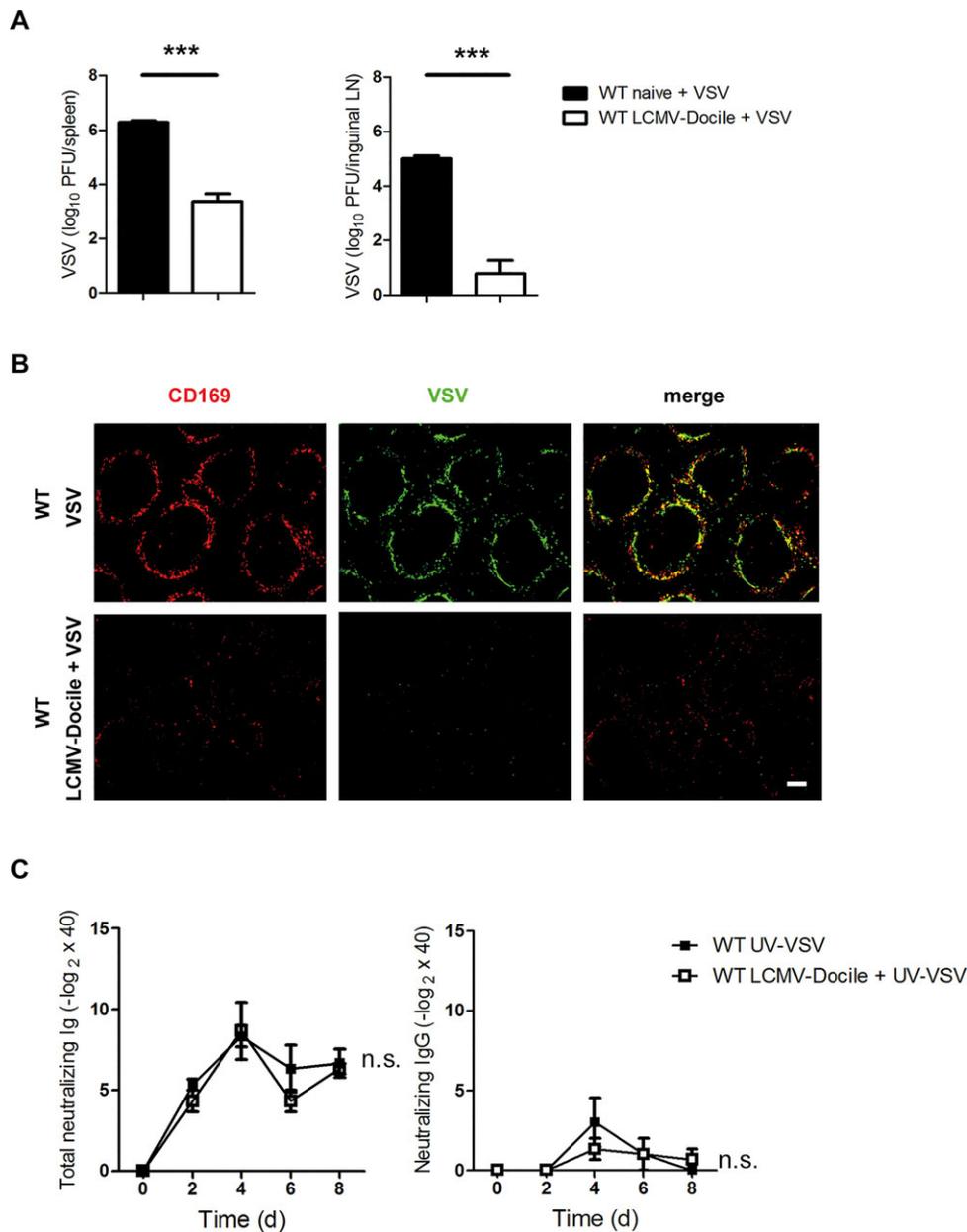
Figure 2. Enforced viral replication is blunted in chronically infected mice. (A) C57BL/6 WT mice were chronically infected with 2×10^4 PFU LCMV strain Docile (LCMV-Docile) for 30 days or left uninfected. In addition, mice were infected with 2×10^8 PFU VSV for 7 h. VSV titers in spleen and inguinal lymph nodes were measured by plaque assay ($n = 3$–6). (B) C57BL/6 WT mice were intravenously infected with 2×10^4 PFU LCMV-Docile for 30 days or left uninfected. Mice were then infected with 2×10^8 PFU VSV for 7 h. Immunofluorescence of spleen sections stained for VSV glycoprotein (green) and CD169 (red). Scale bar = 100 μm ($n = 3$). Fluorescent images were captured at 10x magnification using Keyence BZ-9000E microscope. (C) C57BL/6 WT mice were chronically infected with 2×10^4 PFU LCMV-Docile for 30 days or left uninfected. VSV-neutralizing antibodies were measured in chronically LCMV-infected or uninfected WT mice intravenously immunized with 2×10^8 PFU UV-light inactivated VSV ($n = 3$). (A and C) Data are expressed as means ± SEM and are representative of two independent experiments. .n.s.: not significant; ***$p <$ 0.001. Statistically significant differences were detected with Student's t-test (A) or ANOVA.

mice that were treated with anti-IFN-αR1 antibody in comparison to control WT mice (Fig. 4C). This replication was comparable to WT mice that were infected only with VSV (Fig. 2B). To demonstrate that VSV replication in the absence of the type I IFN effect is sufficient to stimulate the adaptive immune system during chronic infection, we treated LCMV-Docile-infected mice with anti-IFN-αR1 antibody before superinfection with VSV

and measured the production of VSV-neutralizing antibodies. Indeed, blocking of IFN-αR1 prevented immunosuppression and mice showed normal production of neutralizing antibodies comparing to control mice (Fig. 4D). Moreover, we used mice double-deficient in *Irf3* and *Irf7* (*Irf3*$^{-/-}$ × *Irf7*$^{-/-}$), which exhibit a defect in the production of type I IFN [25]. *Irf3*$^{-/-}$ × *Irf7*$^{-/-}$ mice are less susceptible to death after infection with VSV than
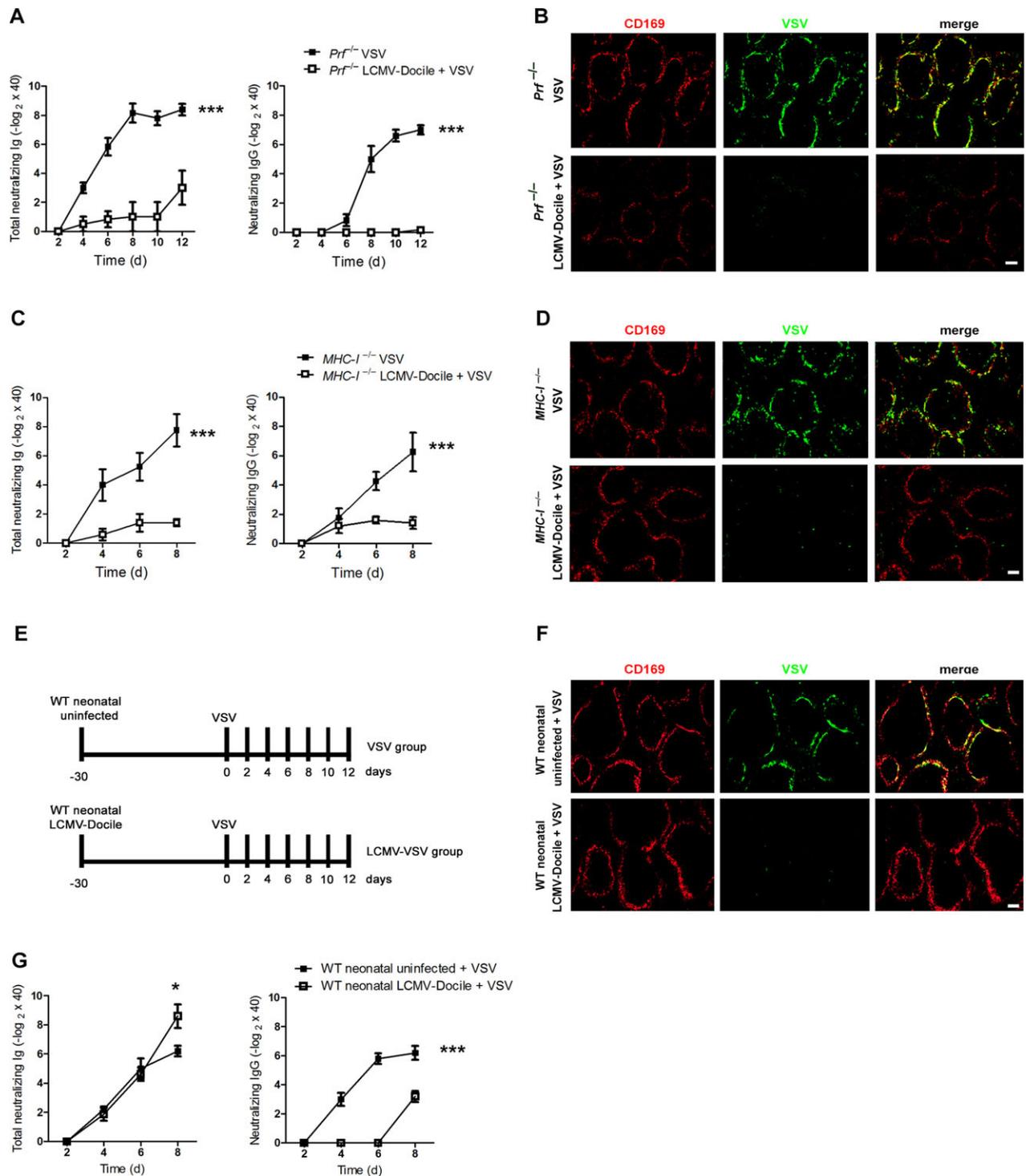
Figure 3. CD8^{+} T cells do not prevent immunosuppression during chronic infection. (A, C, and G): (A) Perforin-deficient ($Prf^{-/-}$), (C) MHC-I-deficient ($MHC-I^{-/-}$), or (G) C57BL/6 neonatal mice were chronically infected with 2×10^4 PFU LCMV strain Docile (LCMV-Docile) or were left uninfected. After 30 days, mice were intravenously infected with 1×10^4 PFU VSV. VSV-neutralizing antibodies were measured by neutralizing assay at the indicated time points ((A and C) $n = 4$–6; (G) $n = 5$–8). Data are expressed as mean $\pm$ SEM and are pooled from two independent experiments. $^*p < 0.5$; $^{***}p < 0.001$. Statistical significance was detected with ANOVA; A, C, and G; IgG) or Student's t-test (G; total Ig). (B, D, and F): (B) $Prf^{-/-}$, (D) $MHC-I^{-/-}$, and (F) neonatal C57BL/6 WT mice were intravenously infected with 2×10^4 PFU LCMV-Docile for 30 days or left uninfected. Mice were then infected with 2×10^8 PFU VSV for 7 h. Immunofluorescence of spleen sections stained for VSV glycoprotein (green) and CD169 (red). Fluorescent images were captured at 10x magnification using Keyence BZ-9000E microscope. Scale bar $= 100 \, \mu$m. Images shown are representative of three independent experiments. (E) Experimental protocol shows infection time points.
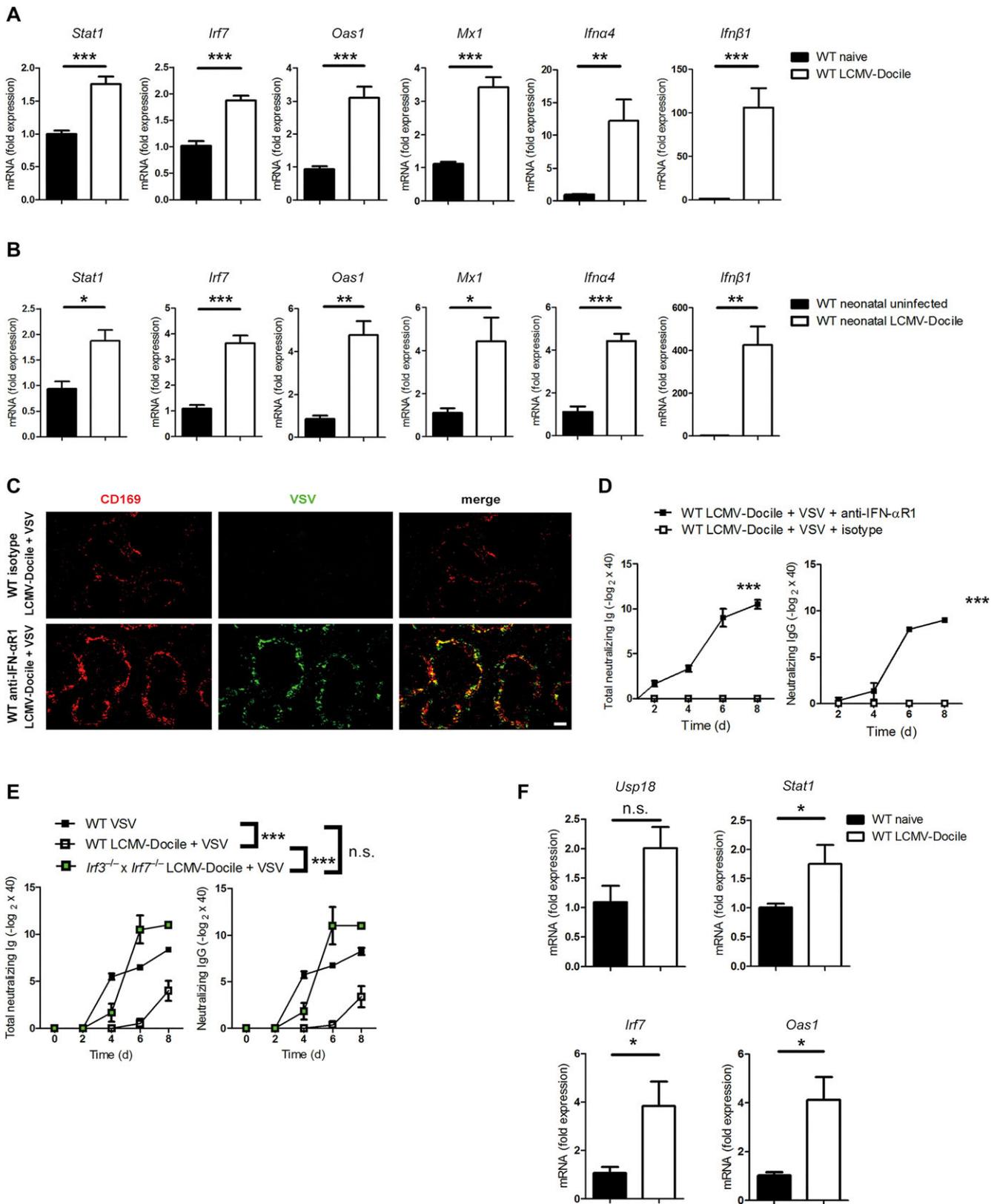
$Ifnar^{-/-}$ mice. We infected $Irf3^{-/-} \times Irf7^{-/-}$ mice with LCMV-Docile and superinfected 30 days later with VSV. The production of neutralizing antibodies against VSV was comparable to that in WT mice that had been infected with VSV alone (Fig. 4E). Therefore, we concluded that chronic viral infection inhibits enforced viral replication by inducing antiviral effector genes. The absence of enforced viral replication limits the adaptive immune response.

In our previous work we found that naive CD169+ macrophages exhibit higher expression of $Usp18$ than do F4/80+ macrophages, and this increase in expression guarantees enforced viral replication [20]. We then questioned why $Usp18$ expression in CD169+ macrophages could not guarantee VSV replication during chronic LCMV infection. We sorted CD169+ macrophages from naive mice and from mice chronically infected with LCMV (Supporting Information Fig. 1) and compared the expression of type I IFN-induced genes by these macrophages with their expression by naive CD169+ macrophages. We found a slight increase in $Usp18$ expression after chronic LCMV infection (Fig. 4F) but a substantial increase in the expression type I IFN-induced genes (Fig. 4F). This finding explains the blunting of enforced viral replication in these cells during chronic infection.

Discussion

In this study, we found that constant antiviral action of type I IFN during chronic infection inhibits the enforced viral replication of a secondary viral infection in specific APCs, such as CD169+ macrophages. The resulting reduction in the level of the antigen limits the activation of the adaptive immune responses against the secondary viral pathogen and consequently leads to immune failure independent of CD8+ T cells. Earlier studies showed that immunosuppression is due to cytotoxic CD8+ T cells [2, 26]. The conclusion was that immunopathological LCMV-specific CD8+ T cells mediate the elimination of LCMV-specific and LCMV-nonspecific infected B cells, and that this elimination prevents the reaction of the immune system against a secondary infection. In the present study we found that early enforced viral replication, which is essential for the activation of the adaptive immune system, is absent in chronically infected mice.

As we showed in a previous study, CD169+ macrophages are essential for enforced viral replication of VSV, and this replication guarantees the activation of the adaptive immune system and the control of the virus [20]. Protecting CD169+ macrophages from the cytotoxicity of CD8+ T cells in $Pfr^{-/-}$ mice or $MHC\text{-}I^{-/-}$ mice did not abrogate either immunosuppression or the reduction in viral replication. Moreover, central deletion of LCMV-specific CD8+ T cells in mice infected as neonates did not prevent immunosuppression, and enforced viral replication was blunted in these mice. Taken together, these findings exclude the hypothesis that CD8+ T cells cause a general immunosuppression in chronically infected mice. However, the prolonged activity of type I IFN during chronic viral infection inhibits the enforced viral replication of a secondary virus and, consequently, inhibits an important mechanism of efficient antigen presentation and activation of the adaptive immune system. In the end, chronic infection leads not to immunosuppression but rather to chronic activation of the immune system.

In conclusion, the results presented here may change our understanding of the reaction of the immune system during chronic infection. Indeed, the failure to generate adaptive immune responses is not due to immunosuppression. Rather, the adaptive immune response is overwhelmed by the ongoing activation of the innate immune system. This condition prevents the secondary virus from replicating efficiently and, in the end, eliminates the virus before it can achieve specific activation of the adaptive immune system. This mechanism may explain why some chronically infected patients do not respond to vaccination. However, additional studies examining the importance of enforced viral replication in achieving successful vaccination are needed.

Materials and methods

Mice and viruses

All experiments were performed with animals housed in single ventilated cages, under the authorization of the Veterinäramt Nordrhein Westfalen (Düsseldorf, Germany) and in accordance with the German law for animal protection. $Prf^{-/-}$ and $MHC\text{-}I^{-/-}$ mice were maintained on a C57BL/6 background. $Irf3^{-/-} \times Irf7^{-/-}$ mice were bred at Charité-University Medicine Berlin, Germany, and were also maintained on a C57BL/6 background. VSV, Indiana strain (VSV-IND, Mudd-Summers isolate), was originally obtained

Figure 4. Prolonged action of type I IFN is responsible for the inhibition of enforced viral replication. (A) C57BL/6 WT mice were infected with LCMV strain Docile (LCMV-Docile) or left uninfected. The expression of indicated genes was measured by quantitative real-time PCR (qRT-PCR) on day 30 after infection ($n = 4$–6). (B) The expression of indicated genes was measured by qRT-PCR 30 days after neonatal C57BL/6 WT mice had been infected with LCMV-Docile or left uninfected ($n = 4$). (C) C57BL/6 WT mice were intravenously infected with 2×10^4 PFU LCMV-Docile for 30 days or left uninfected. Mice were then treated with anti-IFN-αR1 or isotype control antibody and infected with 2×10^8 PFU VSV for 7 h. Immunofluorescence of spleen sections stained for VSV glycoprotein (green) and CD169 (red). Fluorescent images were captured at 10x magnification using Keyence BZ-9000E microscope. Scale bar = 100 μm ($n = 3$). (D) C57BL/6 WT mice were intravenously infected with 2×10^4 PFU LCMV-Docile for 30 days or left uninfected. Mice were then treated with anti-IFN-αR1 or isotype control antibody and infected with 1×10^4 PFU VSV. VSV-neutralizing antibodies were measured at the indicated time points ($n = 3$–6). (E) WT and $Irf3^{-/-} \times Irf7^{-/-}$ mice were chronically infected with LCMV-Docile for 30 days. Control WT mice were left uninfected. Mice were then infected with 1×10^4 PFU VSV. VSV-neutralizing antibodies were measured at the indicated time points ($n = 6$–8). (F) C57BL/6 WT mice were infected with LCMV-Docile or left uninfected. The expression of indicated genes by FACS-sorted CD169+ macrophages was measured by qRT-PCR on day 30 after infection ($n = 3$). (A, B, and D–F) Data are expressed as means ± SEM and are pooled from two independent experiments. n.s.: not significant; $^*p < 0.5$; $^{**}p < 0.01$; $^{***}p < 0.001$. Statistical significance was detected by Student's t-test (A, B, and F) or ANOVA (D and E).

from Prof. D. Kolakofsky (University of Geneva, Switzerland). Virus was propagated on baby hamster kidney 21 cells at an MOI of 0.01 and was then plaqued onto Vero cells [27]. Mice were infected intravenously with VSV (2×10^8 PFU) for histological analysis and plaque assay or with (1×10^4 PFU) for neutralizing antibody measurement. Viral titers were measured with a plaque-forming assay, as previously described. LCMV strain WE was originally obtained from Prof. Dr. F. Lehmann-Grube (Heinrich Pette Institute, Hamburg, Germany), and LCMV strain Docile as gift from Prof. Dr. R. Zinkernagel (University of Zurich, Zurich, Switzerland) was propagated on L929 cells. Mice were infected intravenously with (2×10^4 PFU) LCMV. Neonatal C57BL/6 WT mice were infected intracerebrally 2 days after birth with LCMV-Docile or were left uninfected. After 30 days, WT mice were additionally infected with VSV.

UV-light inactivation

UV-light inactivation of VSV was carried out in 35-mm Petri dishes (Greiner Bio-One, Frickenhausen, Germany) with a 1-mL volume of virus solution (virus concentration, 1×10^9 PFU per mL of Dulbecco's Modified Eagle's Medium with 2% fetal calf serum (DMEM 2% FCS). The virus solution was exposed to a 12-W germicidal lamp (Thermo Scientific, Wilmington, DE, USA) for 10 min at a distance of 10 cm. Mice were infected with 2×10^8 PFU UV-light-inactivated VSV.

Antibody treatment

LCMV-Docile chronic infected mice were treated with anti-IFN-αR1 (clone MAR1-5A3) or mouse IgG1 isotype control antibody (clone MOPC21; Bioxcell, NH, USA) on day 4 (1 mg) and day 2 (500 μg) before VSV infection for histological analysis and additionally on day 5 (250 μg) for neutralizing antibody measurement.

Neutralizing antibody assay

For analysis of IgG kinetics, samples were pretreated with β-mercaptoethanol (0.1 M) for 1 h for removal of IgM and were afterward prediluted (1:40) in DMEM 2% FCS. For analysis of total Ig, serum was directly prediluted (1:40) in medium, after which the complement system was inactivated for 30 min at 56°C. Serum was titrated 1:2 over 12 steps and incubated with 1×10^3 PFU VSV. After 90 min of incubation at 37°C, the virus-serum mixture was plaqued onto Vero cells. An overlay was added after 1 h, and the cells were again incubated at 37°C. Plaques were counted 24 h later by crystal violet staining.

Plaque assay

VSV titers were measured by smashing organs in DMEM 2% FCS. Smashed organs were titrated 1:3 over 12 steps and plaqued onto Vero cells. After 2 h of incubation at 37°C, an overlay was added, and the cells were incubated overnight at 37°C. Plaques were counted 24 h later by crystal violet staining. LCMV titers were measured with a plaque-forming assay using MC57 cells, as previously described [28].

Sorting of CD169+ macrophages

Splenocytes were digested with Liberase DNAse (Roche, Basel, Switzerland) and stained with anti-mSiglec-1 (CD169; R&D Systems, Minneapolis, MN, USA), anti-CD3 (eBioscience, San Diego, CA, USA), anti-CD19 (eBioscience), anti F4/80 (eBioscience), anti-CD11b (eBioscience), and DAPI (Life Technologies, CA, USA). After 30-min incubation, CD169+ cells were sorted by FACS (BD FACS Aria III, BD Biosciences).

Histology

Histological analyses were performed on snap-frozen tissues. Spleen sections were stained for the vesicular stomatitis glycoprotein with the anti-VSV-G antibody (Vi10; made in-house). Metallophilic marginal zone macrophages were stained with mSiglec-1 (CD169, R&D Systems).

Total RNA extraction, cDNA synthesis, and quantitative real-time PCR

RNA was isolated from spleen tissue with the RNA Mini Kit (Qiagen, Hilden, Germany). Quantitation of RNA was performed with a NanoDrop ND-1000 spectrophotometer (Thermo Scientific). The RNA was reverse-transcribed to cDNA with the Quantitect Reverse Transcription Kit (Qiagen). Gene expression analysis was performed with *GAPDH, Stat1, Irf7, Oas1, Mx1, Ifnα4, Ifnβ1,* and *Usp18* assays (Qiagen). For analysis, the expression levels of all target genes were normalized against GAPDH (dCt). Gene expression values were then calculated by the delta-delta-Ct (ddCt) method, with the mean of the control group as the calibrator to which all other samples were compared. Relative quantities (RQs) were determined with the equation $RQ = 2^{-ddCt}$.

Statistical analysis

Unless stated otherwise, data are expressed as means ± SEM. Student's *t*-test was used to detect statistically significant differences between groups. Significant differences between several groups were detected by two-way analysis of variance (ANOVA). The level of statistical significance was set at $p < 0.05$.

FACS sorting was performed by Prof. M. Gunzer, West German Tumor Center (WTZ), at the Imaging Center Essen (IMCES) of University Hospital Essen.

Conflict of interest: The authors declare no financial or commercial conflict of interest.

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Abbreviations: **LCMV**: lymphocytic choriomeningitis virus · **VSV**: vesicular stomatitis virus

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