Rapid Regulation of Depression-Associated Genes in a New Mouse Model Mimicking Interferon-α-Related Depression in Hepatitis C Virus Infection

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Abstract Major depression is a serious side effect of interferon-α (IFN-α), which is used in the therapy of hepatitis C virus (HCV) infection. Due to the lack of reproducible animal models, the mechanisms underlying IFN-α-related depression are largely unknown. We herein established a mouse model, in which murine IFN-α (250 IU/day) and polyinosinic/polycytidylic acid (poly(I:C); 1 μg/day), a toll-like receptor-3 (TLR3) agonist that mimics the effect of HCV double-strand RNA, were continuously infused into the lateral ventricle via miniosmotic pumps over up to 14 days. The delivery of IFN-α and poly(I:C), but not of IFN-α or poly(I:C) alone, resulted in a reproducible depression-like state that was characterized by reduced exploration behavior in open-field tests, increased immobility in tail suspension and forced swimming tests, and a moderate loss of body weight. In the hippocampus and prefrontal cortex, the pro-inflammatory genes TNF-α, IL-6, tissue inhibitor of metalloproteinases-1 (Timp-1), CXC motif ligand-1 (Cxcl1), Cxcl10, and CC motif ligand-5 (Ccl5) were synergistically induced by IFN-α and poly(I:C), most pronounced after 14-day exposure. In comparison, the interferon-inducible genes of signal transducer and activator of transcription-1 (Stat1), guanylate binding protein-1 (Gbp1), proteasome subunit-β type-9 (Psmb9), ubiquitin-conjugating enzyme E2L-6 (Ube2l6), receptor transporter protein-4 (Rtp4), and GTP cyclohydrolase-1 (Gch1), which had previously been elevated in the blood of IFN-α-treated patients developing depression, in the brains of suicidal individuals, and in primary neurons exposed to IFN-α and poly(I:C), were induced even earlier, reaching maximum levels mostly after 24 hours. We propose that interferon-inducible genes might be useful markers of imminent depression.

Keywords Depression · Interferon-α · TLR3 activation · Interferon-inducible genes · Cytokines

Introduction

Although a variety of chronic viral infections and malignant cancers are successfully treated with interferon (IFN)-α, depressive symptoms developing in as many as 30–50 % of
patients receiving IFN-α represent a major burden for patient care. One of the most prevalent diseases treated with IFN-α is chronic hepatitis C virus (HCV) infection, which affects 170 million individuals worldwide, representing the leading cause of progressive liver diseases resulting in hepatic cirrhosis and hepatocellular carcinoma [1]. In HCV patients, as many as 22–31% of patients receiving the standard combination therapy, IFN-α and ribavirin, develop clinically relevant depressive symptoms [2–5]. Interestingly, HCV patients treated with IFN-α present with more severe depressive symptoms than patients treated with IFN-α for other pathologies, such as hepatitis B virus infection, melanoma, or cancer [6–8]. From these observations, it may be concluded that HCV and IFN-α act synergistically on the brain.

IFN-α is a potent trigger of pro-inflammatory cytokines [9, 10], namely of interleukin (IL)-1, IL-6, tumor necrosis factor-α (TNF-α), and IFN-γ, which have been associated with depression [11–14]. Yet, also drug-naïve HCV patients frequently present with fatigue, anxiety, and depressive symptoms [15], most likely as a consequence of pro-inflammatory cytokines induced by HCV [16]. In the brain, toll-like receptor-3 (TLR3), which is expressed on human and murine microglia, astrocytes, and neuronal growth cones [17–20], represents a sensor for HCV that is activated by viral double-strand RNA. TLR3 activation has been reported to induce a very similar pro-inflammatory cytokine response as IFN-α [19–24].

In view of the needs of patients receiving IFN-α for the treatment of HCV infection, we recently examined the effects of murine IFN-α (mIFN-α) and the TLR3 agonist polyinosinic-polycytidylic acid (poly(I:C)) in mouse primary hippocampal and prefrontal cortical neurons [23]. In these neurons, we identified a signature of depression-related interferon-inducible genes (DRIIs) that had been shown to be induced in the blood of depressed HCV patients receiving IFN-α therapy, in the blood of patients with severe major depressive disorder, and in the brain of individuals who had committed suicide [23, 25]. Notably, these genes were most strongly regulated by combined mIFN-α and poly(I:C) exposure, arguing in favor of a synergistic action of both stimuli.

Although depressive-like behavior has been reported following mIFN-α treatment in mice [26, 27], IFN-α alone did not reproducibly result in depressive-like behaviors [28, 29]. Thus, there was a need of an animal model that allows mimicking IFN-α-related depression-like states. Following observations that TLR3 plays a crucial role in anxiety behaviors [30], we now established a mouse model of combined mIFN-α and poly(I:C) exposure, which, as we show, exhibits a reproducible depressive phenotype. In the brains of depressed mice, we now report a signature of DRIIs that is almost identical to the selectively regulated genes in primary mouse neurons and in the brains of individuals who committed suicide [23]. Our animal model provides a promising tool for the evaluation of anti-depressant therapies.

Materials and Methods

Brain Infusion of mIFN-α and Poly(I:C)

Animal experiments were performed with government approval according to the NIH guidelines for the care and use of laboratory animals. C57BL6/J mice were used that were kept in the animal facilities of the University Hospital of Essen in a constant 12 h light/12 h dark regimen. Mice were anesthetized with 1% isoflurane (30% O2, remainder N2O). A total volume of 5 μl of (a) 0.1 M phosphate-buffered saline (PBS; used as vehicle), (b) recombinant mIFN-α (50, 500, 1,000, or 5,000 IU in 0.1 M PBS; Sigma-Aldrich, Deisenhofen, Germany), or (c) poly(I:C) (4, 10, 25, or 100 μg in 0.1 M PBS; tlr-picw, InvivoGen, San Diego, CA, USA) was infused into the left lateral ventricle (1 mm lateral to bregma/2.5 mm below the brain surface) over 5 min either alone or in combination with each other using a Hamilton syringe connected to a stereotoxic injector (Stoelting, Illinois, USA) (n=6 animals per group). Following infusion, the injector was left in place for 2 min to prevent diffusion of chemicals along the needle track. Twenty-four hours later, animals were sacrificed by cervical dislocation in isoflurane anesthesia. Brains were removed and tissue samples were collected from the hippocampi and prefrontal cortices.

In additional experiments, (a) 0.1 M PBS, (b) mIFN-α (250 IU/day in 0.1 M PBS), or (c) poly(I:C) (1 μg/day in 0.1 M PBS) was infused into the left lateral ventricle either alone or in combination with each other using cannulae linked to miniosmotic pumps (Alzet 1002; Palo Alto, CA, USA) (n=12 animals per group, from two independent experiments). Fourteen days later, behavioral assessments were performed as outlined below. Animals were sacrificed by cervical dislocation in isoflurane anesthesia. Brains were removed. Tissue samples were harvested from the hippocampi and prefrontal cortices. Coronal cryostat sections (20 μm thick) were obtained from the levels of the prefrontal cortex (bregma +3.08 to +2.46 mm) and hippocampus (bregma −2.22 to −2.46 mm).

Behavioral Assessment

Behavioral assessment was performed between 10 a.m. and 2 p.m. on day 14 after the initiation of intracerebroventricular mIFN-α or poly(I:C) infusion. Each testing session was arranged in the same order as specified below, with the experimenter blinded for the experimental conditions.
Tests were recorded with a CCD camera. Video recordings were analyzed by two different observers blinded for the experimental groups.

**Open-Field Test** The open-field arena, which had a size of 56×56 cm, was divided into a grid of equally sized areas with a 56×56×50-cm inner area that was surrounded by the outer area. Mice were placed into the center of the chamber and allowed to freely explore it for 20 min. After returning to their cages, animals were allowed to rest at least 10 min before the next test.

**Tail Suspension Test** Mice were suspended 50 cm above the floor by adhesive tape attached approximately 1 cm from the tip of the tail. During a 6-min period, total immobility time was recorded [31]. After returning to their cages, animals were allowed to rest at least 1 h before the next test.

**Forced Swimming Test** During a 6-min period, mice were forced to swim in a round cylinder (diameter 14 cm) filled to 15-cm height with water at room temperature [32]. Throughout the test, animal activity was recorded. During the last 4 min of the test, immobility time was determined. Each mouse was considered immobile when no movements besides those required to keep the head above the water were noticed.

**Gene Expression Measurement by RT-PCR**

RNA extraction was performed from fresh-frozen prefrontal cortical and hippocampal tissue samples using the RNeasy mini kit (Qiagen, Hilden, Germany). Gene expression levels of pro-inflammatory cytokines (TNF-α, IL-6, tissue inhibitor of metalloproteinases-1 [Timp-1], CXC motif ligand-1 [Cxcl1], Cxcl10, and CC motif ligand-5 [Ccl5]) and DRIs (signal transducer and activator of transcription-1 [Stat1], guanylate binding protein-1 [Gbp1], proteasome subunit-β type-9 [Psmb9], ubiquitin-conjugating enzyme E2L-6 [Ube2l6], receptor transporter protein-4 [Rtp4], GTP cyclohydrolase-1 [Gch1], TNF superfamily member 10 [Tnfsf10], myocyte enhancer factor-2a [Mef2a], and dynein light chain type-1 [Dynlct1]) were assessed by real-time PCR (RT-PCR). One-step RT-PCR was carried out with the QuantiTect SYBR Green RT-PCR kit (Qiagen) according to the manufacturer’s instructions [23]. Copy numbers were evaluated in relation to control conditions (vehicle infusion) using β-actin as house-keeping gene. For all genes, commercial primers were used (QuantiTect Primer Assay, Qiagen).

**Western Blot Analysis**

Protein extraction was performed from fresh-frozen prefrontal cortical and hippocampal tissue samples using Ultra Turrax lysis buffer (150 mM NaCl, 1 % Triton X-100, 1 mM EGTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), and EDTA-free protease inhibitor cocktail; Roche, Basel, Switzerland). Homogenates were centrifuged. Protein concentration in supernatants was determined using DC Protein Assay (Bio-Rad Laboratories, CA, USA). Protein samples (20 μg) were separated by SDS-PAGE (Mini-Protean® TGX™ Gels, Bio-Rad Laboratories) at 150 V for 1 h and transferred to polyvinylidene fluoride membranes (Trans-Blot Turbo Transfer Pack Midi format 4-15 %, Bio-Rad Laboratories), which were incubated for 1 h at room temperature in Tris-buffered saline with Tween-20 (TBS-T) (10 mM Tris-HCl [pH 7.6], 150 mM NaCl, and 0.1 % Tween-20) containing 5 % non-fat dry milk. Blocking membranes were incubated overnight at 4 °C with monoclonal rabbit antibodies against Stat1, phosphorylated Stat1 (Ser727), Stat3, phosphorylated Stat3 (Tyr705), extracellular signal-regulated kinase-1/2 (Erk1/2) and phosphorylated Erk1/2 (Thr202/Tyr204) (Cell Signaling; all diluted 1:1,000). After washing with TBS-T, the membranes were incubated with horseradish peroxidase (HRP)-linked goat anti-rabbit IgG secondary antibody (Cell Signaling; diluted 1:3,000) in TBS-T containing 5 % non-fat dry milk. The blots were visualized by enhanced chemiluminescence solution (Amersham ECL Prime Western Blotting Reagents, GE Healthcare, Little Chalfont, UK) using the image acquisition system Fusion FX7 (Vilber Lourmat, Marne-la-Vallée Cedex, France). The blots were subsequently stripped and reincubated with a polyclonal rabbit anti-β-actin antibody that was used as loading control. Blots were densitometrically analyzed using the ImageJ software (NIH, Bethesda, USA).

**Enzyme-Linked Immunosorbent Assay (ELISA)**

TFN-α was detected in protein extracts obtained from fresh-frozen tissue samples using the mouse TNF-α ELISA kit (Biorbyt, Cambridge, UK).

**Immunohistochemistry**

Coronal sections from paraformaldehyde-perfused brains were immersed in blocking solution containing 10 % normal donkey or normal goat serum and 0.3 % Triton X-100 in 0.1 M PBS for 1 h at room temperature, followed by overnight incubation at 4 °C with monoclonal rat anti-glial fibrillary acidic protein (GFAP; Life Technologies, CA, USA; evaluating astrogliaosis), polyclonal goat anti-ionized calcium-binding adaptor molecule 1 (Iba1; Abcam, Cambridge, UK); evaluating microglial reactivity), or polyclonal goat anti-cluster-of-differentiation (CD) 45 antigen (BD Biosciences, New Jersey, USA; evaluating leukocyte infiltration) antibody (all diluted 1:500 in 0.1 M PBS containing 2 % normal donkey or goat serum and 0.3 % Triton X-100). Sections were washed in 0.1 M PBS and incubated with Alexa-488 secondary antibodies (Life Technologies; diluted 1:1,000) and 4′,6-diamidino-2-
phenylindole (DAPI; Sigma-Aldrich) (for GFAP and Iba1 immunostaining) or incubated with biotinylated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, USA; diluted 1:1,000) and 3,3'-diaminobenzidine (DAB) (for CD45 immunostaining). After mounting, brain sections were examined using an ApoTome.2 microscope (Zeiss, Oberkochen, Germany), in which areas exhibiting immunoreactivity were segmented in a standardized way in defined regions of interest (GFAP, Iba1 in hilus of dentate gyrus and medial prefrontal cortex; CD45 in periventricular area adjacent to the hippocampus). For this procedure, identical camera settings and optical density thresholds were applied to all sections for each of the marker proteins. Digitized images were captured with Cell F software (Olympus, Tokyo, Japan). Optical density processing was performed using ImageJ 1.48q software (NIH).

Statistics

Differences between groups were compared using one-way ANOVA followed by Tukey’s post hoc tests. Throughout the study, data were presented as mean±S.E.M. values. p values ≤0.05 were considered significant. Statistical analyses were performed using the GraphPad Prism software (version 4.03).

Results

Identification of Suitable mIFN-α and Poly(I:C) Doses

To determine suitable doses of mIFN-α and poly(I:C) for in vivo experiments, we performed dose-response measurements, evaluating mRNA levels of the interferon-response gene Stat1 following intracerebroventricular mIFN-α and poly(I:C) delivery. Twenty-four hours after infusion, maximum elevations of Stat1 mRNA were noted following 1,000 IU of mIFN-α and 4 μg of poly(I:C) both in the hippocampus and prefrontal cortex (Online Resource 1). Noteworthy, both mIFN-α and poly(I:C) at these doses induced very similar expression levels (around 3×10^5 Stat1 copies normalized to 10^5 β-actin copies). In order to provide an opportunity for the investigation of synergistic effects of both stimuli, the EC25 doses of both (250 IU mIFN-α and 1 μg poly(I:C)) were selected as daily doses for further experiments.

Combined mIFN-α and Poly(I:C) Delivery Induces a Robust Depression-Like State

Delivery of mIFN-α and poly(I:C) induced a robust depression-like behavioral phenotype, reflected by a reproducible reduction of entries into the inner area of the open-field arena (Fig. 1a), a reduction of time spent in the inner area of the open-field arena (Fig. 1b), and a reduction of immobility times in the tail suspension test (Fig. 1c) and forced swimming test (Fig. 1d) after 14 days, which was most pronounced after combined mIFN-α and poly(I:C) exposure. The appearance of depression-like symptoms was associated with a mild loss of body weight that was more pronounced in animals receiving mIFN-α and poly(I:C) (−1.47±0.74 g) than in animals receiving mIFN-α (+0.16±0.41 g) or poly(I:C) (−0.16±0.15 g) only (vehicle-treated control mice +0.70±0.29 g) (Online Resource 2).

Astrocytic and Microglial Responses to mIFN-α and Poly(I:C) Exposure

Since astrocytes and microglia are major sources of inflammatory signals in the brain, we investigated responses of the astrocytic marker GFAP and microglial marker Iba1 following mIFN-α and poly(I:C) exposure in the hippocampus and prefrontal cortex by immunohistochemistry (for regions of interest, see Fig. 2a). Immunoreactivity to GFAP was increased in the hippocampus after 14-day exposure to poly(I:C) and, even more pronounced, combined exposure to mIFN-α and poly(I:C) (Fig. 2b). Similar responses were found in the prefrontal cortex; the changes failed to show significance here (Fig. 2b). In the hippocampus, GFAP + astrocytes were scattered throughout the hilus of the dentate gyrus. In the prefrontal cortex, GFAP + cell were found in close vicinity to the brain surface.

Likewise, immunoreactivity to Iba1 increased within 14 days of exposure to poly(I:C) and mIFN-α plus poly(I:C) in the hippocampus and prefrontal cortex (Fig. 2c). In contrast to GFAP, Iba1+ cells were evenly distributed not only in the hilus of the dentate gyur but also in the prefrontal cortex, where Iba1+ microglia were identified in all cortical layers. Two different types of microglia were observed: While many cells revealed a ramified appearance with moderate Iba1 immunoreactivity, a subset of cells that was more strongly stained had rounded morphology with short processes or lack of processes, thus resembling activated microglia (Fig. 2c).

Combined mIFN-α and Poly(I:C) Exposure Induces Brain Leukocyte Infiltration

By immunohistochemistry, we next examined how mIFN-α and poly(I:C) exposure influenced the infiltration of CD45+ leukocytes in the brain. In mice receiving infusions of vehicle or single infusions of mIFN-α or poly(I:C), only occasional or very few CD45+ leukocytes were noticed in white matter adjacent to brain ventricles after 14-day infusion (Fig. 3). This pattern markedly differed following combined mIFN-α and poly(I:C) exposure, where abundant CD45+ leukocytes were found most often in the vicinity brain ventricles (Fig. 3). Quantification of CD45+ leukocytes revealed that in comparison to animals exposed to vehicle, to mIFN-α alone, or to poly(I:C) alone, animals exposed to mIFN-α and poly(I:C)
revealed ∼178, ∼62, and ∼18 times higher leukocyte density (Fig. 3). Thus, with respect to leukocyte infiltrates, the condition of combined mIFN-α and poly(I:C) exposure significantly differed from all other three conditions. In the prefrontal cortex, CD45+ leukocytes were only rarely found.

Regulation of Pro-inflammatory Cytokines, Chemokines, and Proteases by mIFN-α and Poly(I:C)

We next evaluated the expression of the pro-inflammatory molecules TNF-α, IL-6, Timp-1, Cxcl1, Cxcl10, and Ccl5 by RT-PCR in the hippocampus and prefrontal cortex after 24 h and 14 days of mIFN-α and poly(I:C) exposure. The expression of all genes except Timp-1, which was strongly elevated in the hippocampus already after 24 h, was considerably higher after 14 days than 24 h (Fig. 4). While mIFN-α alone did not induce any major alterations in the level of pro-inflammatory molecules, poly(I:C) exposure and, more strongly, combined mIFN-α and poly(I:C) exposure increased the expression of all six genes (Fig. 4). Responses of IL-6 to mIFN-α and poly(I:C) were stronger in the prefrontal cortex than in hippocampus, while responses of Ccl5 were stronger in the hippocampus than in prefrontal cortex. Further experiments employing ELISA revealed that the combined delivery of mIFN-α and poly(I:C) for 14 days indeed increased the protein concentration of TNF-α in the hippocampus (Fig. 5).

Regulation of Depression-Related Genes by mIFN-α and Poly(I:C)

In contrast to pro-inflammatory cytokines and chemokines, most of which exhibited a delayed expression maximum, the DRIIs Stat1, Gbp1, Psmb9, Ube2l6, Rtp4, and Gch1, which have previously been shown to be induced by mIFN-α and poly(I:C) in primary hippocampal and prefrontal cortical neuronal cultures [23], were more strongly regulated already after 24 h, as revealed by RT-PCR (Fig. 6). Except for Stat1 and Rtp4, which were elevated in the hippocampus and prefrontal cortex after 24 h but 14 days, mIFN-α alone did not induce any major alterations in DRII expression (Fig. 6). Conversely, poly(I:C) exposure and, more potently, combined mIFN-α and poly(I:C) exposure induced Stat1, Gbp1, Psmb9, Ube2l6, and Rtp4 in the hippocampus and Stat1 and Rtp4 in the prefrontal cortex after 24 h (Fig. 6). These gene responses persisted after 14 days, but at this later time point, expression levels after poly(I:C) delivery resembled those after combined mIFN-α and poly(I:C) delivery (Fig. 6). An interesting observation was a delayed induction of Gch1 after 14 days of combined mIFN-α and poly(I:C) exposure that was significant in the hippocampus but not in the prefrontal cortex (Fig. 6). Tnfsf10, Mef2a, and Dynlct1, which have previously been found to be regulated by mIFN-α and poly(I:C) in primary neuronal cultures [23], did not reveal any significant expression changes in response to mIFN-α and poly(I:C) in mice (Online Resource 3).
Role of Stat1 and Stat3 in mIFN-α and Poly(I:C) Signaling

Since Stat1, Stat3, and Erk1/2 have previously been involved in the regulation of DRIIs in primary hippocampal and prefrontal cortical neuronal cultures, we finally examined their abundance and phosphorylation, i.e., activation by Western blots. As depicted in Fig. 7, combined exposure to mIFN-α and poly(I:C) for 14 days increased the overall abundance of Figs. 2

Combined mIFN-α and poly(I:C) exposure induces astrocytic and microglial immunoreactivity. a Regions of interest (hilus of the dentate gyrus and medial prefrontal cortex), in which immunoreactivity was semiquantitatively analyzed using an observer-independent unbiased densitometry approach, b immunoreactivity for the astrocytic marker GFAP in both regions of interest, evaluated in the hilus of the dentate gyrus (upper) and medial prefrontal cortex (lower), and (c) immunoreactivity for the microglial marker Iba1 in both regions of interest, as evaluated in the hilus of the dentate gyrus (upper) and medial prefrontal cortex (lower) after 14-day exposure to mIFN-α (250 IU/day), poly(I:C) (1 μg/day), or both. Representative microphotographs are shown, in addition to optical densitometric analyses. Arrowheads in (c) point at microglial cells exhibiting features of activated microglia (intense staining, rounded cell body, and short or absent cell processes). Data are mean values±S.E.M., evaluated by one-way ANOVA followed by Tukey’s post hoc tests (n=6 animals per group). *p≤0.05/**p≤0.01/***p≤0.001 compared with vehicle, #p≤0.05/##p≤0.01/###p≤0.001 compared with mIFN-α or poly(I:C) only (as indicated). Scale bar, 100 μm.
Stat1 in the hippocampus and prefrontal cortex and furthermore increased the phosphorylation, i.e., activation of Stat1 in the prefrontal cortex. While the overall abundance of Stat3 did not change in response to mIFN-α and poly(I:C), an increased phosphorylation, i.e., activation of Stat3, was noticed in the hippocampus after combined mIFN-α and poly(I:C) exposure. Erk1/2 and phosphorylated, i.e., activated, Erk1/2 did not show any changes in both brain structures following mIFN-α and poly(I:C) delivery (data not shown).

Discussion

We report a novel mouse model of IFN-α-related depression, in which mIFN-α and the TLR3 agonist poly(I:C) are continuously delivered to the brain by means of miniosmotic pumps. In this model, we observed that the combined delivery of IFN-α and poly(I:C), but not IFN-α or poly(I:C) delivery alone, induced a reproducible depression-like state that was characterized by (a) reduced entries into the inner area and reduced time spent in the inner area in open-field tests (thus reflecting reduced exploration behavior), (b) increased immobility in tail suspension and forced swimming tests, and (c) a moderate loss of body weight. Using immunohistochemistry, RT-PCR, and ELISA, we showed that the delivery of IFN-α and poly(I:C) synergistically evoked a pro-inflammatory state characterized by astrocytic reactivity, microglial activation, and leukocyte infiltration, in which the pro-inflammatory cytokines, chemokines, and proteases TNF-α, IL-6, Timp-1, Cxcl1, Cxcl10, and Ccl5 were overexpressed in the hippocampus and prefrontal cortex together with a signature of six DRIIs, namely, Stat1, Gbp1, Psmb9, Ube2l6, Rtp4, and Gch1, which had previously been shown to be induced in the blood of HCV patients developing depression during IFN-α therapy, in the blood of patients with severe major depressive disorder, in the brain of individuals who had committed suicide, and in primary neurons exposed to IFN-α and poly(I:C) [23, 25]. Notably, the majority of these genes, namely, Stat1, Gbp1, Psmb9, Ube2l6, and Rtp4, reached maximum levels already 24 h after IFN-α and poly(I:C) exposure, which was before the peak of the above cytokines and chemokines. Our results argue in favor of an early immune response during IFN-α exposure, which sets the stage for the subsequent development of depression [33–35]. We propose that DRIIs might be useful markers of imminent depression.

Although depressive-like behavior has previously been reported following IFN-α delivery in mice and rats [26–29, 36, 37], IFN-α alone did not reproducibly result in depressive-like behaviors [28, 29]. This raised questions about the utility of rodent models for evaluating IFN-α-related depression. In clinics, IFN-α is usually delivered to patients suffering from inflammatory states, either related to chronic infection (such as HCV infection) or malignant tumors, but not to healthy subjects. We herein used the TLR3 agonist poly(I:C) for mimicking the effects of viral double-strand RNA on the brain. Our model assumes that during HCV infection, at least some virus or viral constituents get access to the brain, which

Fig. 3 Combined mIFN-α and poly(I:C) exposure induces periventricular leukocyte infiltration. Immunoreactivity for the leukocyte marker CD45, as evaluated in the most anterior hippocampus adjacent to the lateral ventricles after 14-day exposure to mIFN (250 IU/day), poly(I:C) (1 μg/day), or both. Representative microphotographs are shown, in addition to unbiased analyses evaluating the area covered by leukocytes in comparison to the whole area of interest (labeled “PV” [stands for “periventricular”] in the microphotographs). Data are mean values±S.E.M., evaluated by one-way ANOVA followed by Tukey’s post hoc tests (n=6 animals per group). **p≤0.01/****p≤0.001 compared with vehicle, #p≤0.05/###p≤0.001 compared with mIFN-α or poly(I:C) only (as indicated). Scale bar, 100 μm.
is supported by experimental observations of HCV neuroinvasion and CNS replication in more than half of patients exhibiting the phase of viremia [38, 39]. In our study, the combined exposure to mIFN-α and poly(I:C), but not to mIFN-α or poly(I:C) alone, induced a reproducible depression-like state, in line with earlier studies [28, 29]. Our data emphasize the importance of an appropriate pro-inflammatory milieu for the development of depressive symptoms. Both mIFN-α and poly(I:C) were well tolerated by mice. Only very mild changes in body weight were noted when mIFN-α or poly(I:C) alone was administered. This observation argues against major side effects of the two stimuli, such as sickness behavior, which has previously been reported after bolus injections of IFN-α [27, 37, 40, 41]. There is growing evidence from animal models and human patients that systemic and CNS inflammation predisposes to depression [14, 42, 43]. Thus, we evaluated histopathological sequelae of brain inflammation following mIFN-α and poly(I:C) exposure. By means of immunocytochemical studies, we show that the development of depressive symptoms
following mIFN-α and poly(I:C) delivery involves synergistic responses of astrocytes and microglia, reflected by GFAP and Iba1 expression, and the brain recruitment of CD45+ leukocytes. In this study, mIFN-α and poly(I:C) were intracerebroventricularly administered, achieving relatively high local concentrations in the vicinity of the lateral ventricles. Yet, HCV exhibits a strong tropism to astrocytes and microglial cells, and blood-derived leukocytes are also known to accumulate in the brain during the CNS invasion of HCV [39, 44]. Thus, our model might reflect the histopathological sequelae of HCV infection in a realistic and appropriate way.

At the molecular level, mIFN-α and poly(I:C) synergistically increased TNF-α, IL-6, Timp-1, Cxcl1, Cxcl10, and Ccl5 levels in the hippocampus and prefrontal cortex. Elevated levels of IL-6, Timp-1, and Cxcl9 have previously been described in the blood of HCV patients developing depression during IFN-α therapy, in the blood of patients with severe major depressive disorder, and in the brains of subjects who committed suicide [23, 25]. Elevated levels of TNF-α, IL-6, Timp-1, Cxcl1, Cxcl10, and Ccl5 have also been shown in primary mouse hippocampal and prefrontal cortical neurons exposed to mIFN-α and poly(I:C) [23]. In human patients suffering from major depression, elevated blood concentrations of TNF-α and IL-6 have repeatedly been reported [45–48]. Based on the common pattern of regulation in vitro, in vivo, and in human patients, we suggest that TNF-α, IL-6, Timp-1, Cxcl1, Cxcl10, and Ccl5 represent a common genetic signature that unravels links between brain inflammation and depression pathogenesis.

While the regulation of cytokines and chemokines TNF-α, IL-6, Timp-1, Cxcl1, Cxcl10, and Ccl5 was most pronounced after 14 days of mIFN-α and poly(I:C) exposure, we were surprised to see that the DRIIs Stat1, Gbp1, Psmb9, Ube2l6, Rtp4, and Gch1 were induced even earlier, reaching maximum levels at 24 h in the case of Stat1, Gbp1, Psmb9, Ube2l6 and Rtp4. Similar to the above cytokines and chemokines, the
mRNAs of these six DRIIs have also been increased in the blood of HCV patients developing depression during IFN-α therapy, in the blood of patients with severe major depression, in the brains of individuals who committed suicide, and in primary mouse hippocampal and prefrontal cortical neurons exposed to mIFN-α and poly(I:C) [23, 25]. In the blood of depressed HCV patients receiving IFN-α therapy, in the blood of patients with major depression, in brains of suicidal subjects, and in primary neurons exposed to mIFN-α and poly(I:C), we have previously also observed elevated Tnfsf10, Mef2a, and Dynlt1 expression [23, 25], which we could not confirm in this study. A number of observations support a role of DRIIs in depression pathogenesis. Stat1 encodes a central mediator of type I and II IFN actions, which promotes apoptosis and impairs neuronal survival [49], thus contributing to the cytokine neurotoxicity [50]. It has been reported that Stat1 to a certain extent can activate Stat3, although in a more transient fashion [51], and that the balance between Stat1 and Stat3 might determine neuronal survival after nerve injury [49]. Besides, Stat1 was found to deregulate neurotransmitter systems, notably 5-hydroxytryptamine (5-HT), dopamine, and glutamate, which are involved in the pathogenesis of major depressive disorder [52]. Gbp1 encodes an intracellular regulator of the proliferation and migration of endothelial cells in response to pro-inflammatory cytokines, which is detected in the serum of patients with chronic inflammatory disorders [53]. Psmb9 and Ube2l6 encode members of the ubiquitin-proteasome system, which have been previously found to be induced by Stat1 following exposure to IFN-α and oncostatin M, a member of the IL-6 cytokine family [54]. Gch1 encodes the rate-limiting enzyme of GTP conversion into 7,8-dihydroneopterin (BH2), thus increasing the formation of neopterin at the expense of tetrahydrobiopterin (BH4), which is a rate-limiting enzyme in 5-HT and dopamine biosynthesis [55, 56]. Links between depression related to inflammation, neurotransmitter systems, and neuronal survival and death have recently been comprehensively reviewed [57].

In view of their rapid regulation, we hypothesize that DRIIs might represent an early surrogate of imminent depression. Further studies will be needed to determine whether DRII induction also precedes the development of depression in HCV-infected patients receiving IFN-α. In this case, DRII
might be used for therapeutic monitoring. Yet, in view of the elevated expression of DRII in the blood of patients suffering from major depressive disorder [25], our observations might have consequences reaching far beyond IFN-α-related depression. Thus, our animal model might offer itself for the evaluation of depression pathogenesis and may represent a tool for the assessment of anti-depressant therapies.

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Conflict of Interest  The authors declare that they have no conflict of interest.

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