IL-28A and IL-29 mediate antiproliferative and antiviral signals in intestinal epithelial cells and murine CMV infection increases colonic IL-28A expression

Stephan Brand,1* Florian Beigel,1,2 Torsten Olszak,1 Kathrin Zitzmann,1 Sören T. Eichhorst,1 Jan-Michel Otte,2 Joachim Diebold,3 Helmut Diepolder,1 Barbara Adler,4 Christoph J. Auernhammer,1 Burkhard Göke,1 and Julia Dambacher1

1Department of Medicine II, University-Hospital Munich-Grosshadern, University of Munich, Munich; 2Department of Medicine I, St. Josef-Hospital, Ruhr-University, Bochum; 3Institute of Pathology, University-Hospital Munich-Grosshadern, University of Munich, Munich; and 4Max von Pettenkofer-Institute, University of Munich, Munich, Germany

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ACUTE GASTROENTERITIS is one of the most common illnesses, affecting both adults and children. Several groups of viruses are responsible for a large proportion of these infections, including rotaviruses, enteric adenoviruses, human calciviruses (the prototypic example being noroviruses or Norwalk-like viruses), and astroviruses. Moreover, human cytomegalovirus (HCMV) is a major cause of morbidity and mortality in immunocompromised individuals. During severe HCMV disease in patients with acquired immunodeficiency syndrome (AIDS), the gastrointestinal tract is frequently involved and typically shows mucosal lesions resembling features of inflammatory bowel disease. Although the current therapy of viral gastroenteritis is mostly supportive, several antiviral agents are currently under investigation for other applications. Among these agents are type I interferons (IFNs), which show also antiviral properties against HCMV infection (5).

Several novel interleukin (IL)-10-related cytokines have recently been discovered. These include IL-22, IL-26, and IFN-λs. Because IFN-λ proteins activate IFN-stimulated response elements and induce antiviral activity, these cytokines have been considered to be a novel group of “IFNs” (23). The IFN-λ gene family is composed of three distinct genes: IFN-λ1 (IL-29), IFN-λ2 (IL-28A), and IFN-λ3 (IL-28B) (23, 32). IFN-λ proteins exhibit only weak homology to IL-10, but, like IL-10, they also use the IL-10R2 chain as a component of their receptor complex. The IFN-λ receptor complex consists of the unique ligand-binding chain IFN-λR (also known as IL-28R) and the accessory receptor chain IL-10R2. Whereas signaling through IL-22R and IL-26R complexes results predominantly in the activation of signal transducer and activator of transcription (STAT)3, activation of STAT1 and STAT2 is detected in the activation of signal transducer and activator of transcription (STAT)3. Site-directed mutagenesis of STAT1, STAT2, or STAT3 inhibits IFN-λR signaling, whereas site-directed mutagenesis of STAT1 or STAT2, or STAT3 does not affect IFN-λR signaling (23, 32). Although virtually any cell type after viral infection can express IFN-λ, dendritic cells appear to be major producers of IFN-λ (9). IFN-λRs are expressed at variable levels on most cell types. Signaling through IFN-λRs results in the induction of many of the same genes that are induced by signaling through IFN-α/βRs such as antiviral proteins (23). Viruses are potent inducers of the expression of type I IFNs. IFN-α is particularly expressed by cells of a lymphoid origin, whereas IFN-β is expressed by most cell types (17). It is believed that double-stranded RNA (dsRNA) might be the

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* S. Brand and F. Beigel contributed equally to this work.

Address for reprint requests and other correspondence: S. Brand, Dept. of Medicine II-Grosshadern, Univ. of Munich, Marchioninistrasse 15, D-81377 Munich, Germany (e-mail: stephan.brand@med.uni-muenchen.de).

First published July 28, 2005; doi:10.1152/ajpgi.00126.2005.—Human cytomegalovirus virus (HCMV) is a major cause of morbidity and mortality in immunocompromised individuals. Recently, a novel group of cytokines [interleukin (IL)-28A/B and IL-29, also termed interferon (IFN)-λs] has been described. Here, we demonstrate that intestinal epithelial cell (IEC) lines as well as murine and human colonic tissue express the IFN-λ receptor subunits IL-28R and IL-10R2. IL-28A and IL-29 binding to their receptor complex activates ERK-1/2 and stress-activated protein kinase/c-Jun NH2-terminal kinase MAPKs (JNK) and Akt, resulting in increased IL-8 protein expression. IFN-λs also induce phosphorylation of signal transducer and activator of transcription 1 and significantly increase mRNA expression of suppressor of cytokine signaling 3 and the antiviral proteins myxovirus myristovirus resistance A and 2',5'-oligoadenylate synthetase. These signals result in an up to 83% reduction of cells positive for human CMV immediate-early (IE) genes are coexpressed to-
common mechanism by which viruses induce expression of IFNs. The synthetic polymer poly I:C is widely used to induce IFNs. The IFN-λs IL-28A/B and IL-29 have also been shown to be expressed by human peripheral blood mononuclear cells after infection with encephalomyocarditis virus (EMCV) or after stimulation with poly I:C (23, 32). This may indicate that type I IFNs and the IL-28/IL-29 subfamily have common regulatory elements and similar biological effects. For example, IL-28 and IL-29 have been reported to protect human cell lines against the cytopathogenic effect of EMCV or vesicular stomatitis virus (23, 32), indicating potent antiviral activity. However, the individual roles of type I IFNs and IL-28/IL-29 in antiviral responses, particularly in intestinal epithelial cells (IECs), remain to be established.

In this study, we demonstrated that IECs express the IFN-λ/ receptor complex. Both IL-28A and IL-29 efficiently activate STAT1, inducing the expression of antiviral proteins and strong inhibition of HCMV protein expression after infection. IL-28A mRNA expression was increased after murine CMV (MCMV) infection in vivo. Furthermore, IL-28A and IL-29 significantly decreased the proliferation of IECs in vitro. Therefore, IFN-λ cytokines may have therapeutic potential in the treatment of certain viral infections and, considering their antiproliferative effects, as anticancer therapy.

MATERIALS AND METHODS

Reagents. Polyclonal antibodies specific for phosphorylated (phospho)-ERK1-2 (Thr183/Tyr185), phospho-stress-activated protein kinase (SAPK)/c-Jun NH2-terminal kinase (JNK) (Thr183/Tyr185), phospho-p38 (Thr180/Tyr182), and phospho-Akt (Ser473) were purchased from Cell Signaling (Beverly, MA). Anti-ERK-1/2, anti-SAPK/JNK, anti-p38, and anti-Akt antibodies were also from Cell Signaling. Antibody against phospho-STAT1, which recognizes the two splicing variants of STAT1, was purchased from BD Transduction Laboratories (Franklin Lakes, NY), and antibody against phospho-STAT3 was from Upstate Biotechnology (Lake Placid, NY). Antibodies against STAT3 and STAT1 were from Santa Cruz Biotechnology (Santa Cruz, CA). Horseradish peroxidase-linked anti-rabbit secondary antibody was purchased from Amersham (Arlington Heights, IL). Recombinant human IL-28A and IL-29 were obtained from R&D Systems (Minneapolis, MN). The MEK-1 inhibitor PD-98059, the p38 inhibitor SB-203580, and the SOCS-3 antibody were purchased from Tocris Cookson (Bristol, UK).

Cell culture. The human colorectal cancer-derived cell lines Caco-2, DLD-1, SW480, HCT116, and HT-29 and the primary epithelial cell line Intestine-407 (Int-407; American Type Culture Collection CCL6), derived from the human embryonic jejunum and ileum, were obtained from American Type Culture Collection (Rockville, MD) and grown in DMEM (GIBCO-BRL; Gaithersburg, MD) with 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10% heat-inactivated FCS (PAA; Pasching, Austria) in a humidified 5% CO2 atmosphere at 37°C. For signal transduction experiments with IL-28A and IL-29, cells were starved overnight in serum-free medium.

RT-PCR. Total RNA was isolated from cell lines and human and murine colonic tissue using TRIzol reagent (GIBCO-BRL). Human colonic tissue was taken from patients undergoing routine diagnostic colonoscopy. The study was approved by the Ethics Committee of the Medical Faculty of the University of Munich and adhered to the principles of the Declaration of Helsinki as well as to Title 45, United States Code of Federal Regulations, Part 46, Protection of Human Subjects, Revised November 13, 2001, effective December 13, 2001. All participating subjects gave written informed consent before the colonoscopy. For RT-PCR, RNA was treated with RNase-free deoxyribonuclease (DNA-Free Kit, Ambion) to remove potential genomic DNA contaminants. Three micrograms of total RNA were reverse transcribed using a Roche first-strand cDNA synthesis kit. To control for genomic contamination, an identical parallel PCR was performed containing starting material that had not been reverse transcribed. The following conditions were used for semiquantitative PCRs: denaturing at 95°C for 45 s, annealing at 61°C for 45 s, and extension at 72°C for 45 s. All PCRs included GADPH primers to quantify PCR products. The primers for the PCRs are shown in Table 1. All primers were designed not to amplify genomic DNA. Previously published primers were used for the PCRs for PKR, myxovirus resistance A (MxA), and 2’-5’-oligoadenylate synthetase (2’-5’-OAS) (35). The PCR products were subcloned into pCR 2.1 vector (Invitrogen, Carlsbad, CA) and sequenced.

ELISA. For the quantification of IL-8 release, a BD OptEIA Human IL-8 Elisa Kit II (BD Biosciences; Bedford, MA) was used according to the manufacturer’s instructions.

Cell proliferation assay. HCT116 and HT-29 cells were seeded onto 96-well plates at a density of 5,000 cells/well and grown for 1 day. After starvation in serum-free medium overnight, cells were stimulated with 10, 100, or 1,000 ng/ml IL-28A or IL-29 in serum-free medium as indicated or with cytokine-free and serum-free medium (negative control). The cell proliferation rate was determined by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium assay after 48 h using the CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay (Promega; Madison, WI).

Table 1. PCR primers used for IL-10R2, IL-28R, IL-28A, SOCS-3, and GADPH amplification

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Human</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10R2</td>
<td>Forward 5'-GGCTGAATTTTGCAAGATGACA-3'</td>
<td>Forward 5'-GGCCCTAATACCTGTTCTGCTGT-3'</td>
</tr>
<tr>
<td>IL-10R2</td>
<td>Reverse 5'-GAAACCAAGGAGCATGGG-3'</td>
<td>Reverse 5'-GGACCAACAGGAGCATGGG-3'</td>
</tr>
<tr>
<td>IL-28R</td>
<td>Forward 5'-ACCTATTATTGTCAGCAGCTATG-3'</td>
<td>Forward 5'-TTCTGATCCGCGACAGCTC-3'</td>
</tr>
<tr>
<td>IL-28R</td>
<td>Reverse 5'-CGGCCTGACATTCCAAAAGGTAAT-3'</td>
<td>Reverse 5'-TGCAAAGAAGAGCTGGCC-3'</td>
</tr>
<tr>
<td>SOCS-3</td>
<td>Forward 5'-GGACGTCAAAGGATTTGCTCTGAT-3'</td>
<td>Reverse 5'-AGCCCTTTGGCCTATGGTGAGACG-3'</td>
</tr>
<tr>
<td>GADPH</td>
<td>Reverse 5'-AGCCCTTTGGCCTATGGTGAGACG-3'</td>
<td>reverse 5'-TCTCCTATAGGCTGGAGACA-3'</td>
</tr>
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IL-10R2, interleukin (IL)-10 receptor 2; IL-28R, IL-28 receptor; SOCS-3, suppressor of cytokine signaling 3.
After 45 h, mice were euthanized by CO2 asphyxiation, and the large strain (30) in PBS. Control mice received an injection of PBS only.

**RESULTS**

**HCMV infection in vitro assay.** HCT116 cells, which showed the highest HCMV infection rate in pilot experiments, were grown in 24-well plates to 40–50% confluency. Next, cells were incubated with 10 and 100 ng/ml IL-28A or IL-29 in medium containing 5% FCS or with cytokine-free medium (negative control) for 24 h before infection. The cytokine-containing medium was removed, and cells were infected with HCMV at a multiplicity of infection of 2 in fresh medium containing 5% FCS. Two hours after infection, medium was removed, and cells were incubated with fresh medium containing IL-28A or IL-29 for 72 h. Infection was followed by indirect immunofluorescence using a murine antibody directed against HCMV immediate-early protein (72 kDa, Perkin-Elmer) and an anti-mouse antibody (Sigma; Taufkirchen, Germany). Experiments were made in duplicate wells, and, for each well, positive cells in five random visual fields were counted.

**MCMV infection in vivo.** C57BL/6 mice were infected intravenously with 1 × 10^7 plaque-forming units of MCMV of the Smith strain (30) in PBS. Control mice received an injection of PBS only. After 45 h, mice were euthanized by CO2 asphyxiation, and the large intestine was collected. Total RNA of the colon was isolated using TRIzol reagent. This study was approved by the Animal Care and Use Committee of the State of Bavaria (Regierung von Oberbayern) following the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Statistical analysis.** Statistical analysis was performed using a two-tailed Student’s t-test. P levels <0.05 were considered as significant.

**The IL-28R complex is expressed in IECs.** To determine whether the IL-28R complex, consisting of IL-10R2 and IL-28R, is expressed in IECs and to utilize an IEC model to study this ligand-receptor system, we analyzed IL-10R2 and IL-28R mRNA expression in several human colorectal cancer-derived IEC lines (Caco-2, DLD-1, SW480, HCT116, and HT-29). RT-PCR analysis demonstrated IL-10R2 and IL-28R mRNA expression in all cell lines tested (Fig. 1A). Because IL-28R and IL-10R2 were expressed in all colorectal cancer cell lines tested, we also performed RT-PCR analysis in several other cancerous cell lines derived from different organs including the pancreatic carcinoma cell lines Panc-1 and Mia-Pa-Ca-2, the prostate cancer cell line LNCaP, the hepatoma cell line HepG2, the melanoma cell line 624.38-MEL, and the breast cancer cell lines MCF-7 and SkBr3. LNCaP and 624.38-MEL were the only cell lines that did not express IL-28R (Fig. 1A and data not shown).

To determine whether IL-10R2 and IL-28R are also expressed in noncancerous cells, we additionally analyzed receptor expression in human colonic tissue and in the primary IEC line Int-407 (American Type Culture Collection CCL6), which is derived from the human embryonic jejunum and ileum. IL-28R mRNA was also found in normal human colonic tissue (Fig. 1B) and the untransformed cell line CCL-6 (Fig. 1A).

The two IFN-γs IL-28A and IL-29 induce ERK-1 and ERK-2 activation by a MEK-1-dependent mechanism. Having demonstrated that both subunits required for IFN-γ signaling are expressed in IECs, we next investigated whether the IFN-γR complex is functional in IECs. Therefore, we analyzed which signaling pathways are activated by IL-28A and IL-29. Both IL-28A and IL-29 (100 ng/ml each) induced a transient activation of ERK-1/2 in HT-29 cells (Fig. 2). During the observed time interval, total ERK-1 and ERK-2 levels remained unchanged. To identify the upstream signaling events, we investigated the effect of the MEK-1 inhibitor PD-98059 on IL-28A- and IL-29-mediated ERK phosphorylation. PD-98059 downregulated IL-28A- and IL-29-induced ERK-1/2 phosphorylation (Fig. 2), suggesting MEK-1 as an upstream signal transducer of IL-28A- and IL-29-induced ERK activation.

**IL-28R activation results in SAPK/JNK and Akt phosphorylation.** Because activation of class II cytokine receptors may also result in activation of p38 and SAPK/JNK MAPKs (26), we analyzed these signaling pathways in IECs stimulated with IFN-γs. Stimulation of HT-29 cells with IL-28A and IL-29 resulted in phosphorylation of SAPK/JNK MAPKs, which was inhibited by pretreatment with the JNK inhibitor SP-600125 (Fig. 3). However, IFN-γs did not significantly increase phosphorylation levels of p38 (data not shown). Furthermore, IL-28A and IL-29 also increased phosphorylation of Akt (Fig. 4). Pretreatment with the PI3K inhibitor wortmannin inhibited this phosphorylation (Fig. 4), suggesting a PI3K-dependent pathway.

**IFN-γ increase IL-8 protein expression in IEC.** Activation of MAPKs, particularly ERK and SAPK/JNK MAPKs, has been shown to increase IL-8 expression in epithelial cells (27). Moreover, proinflammatory functions were demonstrated for IL-10-related cytokines such as IL-22 (4). Therefore, we analyzed the influence of IL-28A and IL-29 on IL-8 production in...
the IEC line HT-29. As shown in Fig. 5, both cytokines clearly increased IL-8 protein expression (IL-28A: 2.6-fold and IL-29: 3.6-fold).

IFNs do not influence Fas ligand-induced apoptosis but decrease cell proliferation in IECs. We demonstrated that stimulation of IECs with IL-28A and IL-29 results in ERK-1/2 and Akt activation, which has been shown to mediate antiapoptotic pathways and increase cell proliferation (11, 16); features that are found, for example, in cancerous tissue resulting in enhanced tumor growth and resistance to “apoptotic” and antiproliferative therapy strategies (13). Therefore, we investigated IFN-λ-mediated effects on apoptosis using previously established experimental conditions (14). SW480 cells, which are less resistant to Fas-induced apoptosis than HT-29 cells (1), were used in these assays. However, no significant difference in the number of apoptotic cells between the IL-28A- and IL-29-stimulated and unstimulated group was found (Fig. 6A). In contrast, IL-28A and IL-29 decreased cell proliferation in HCT116 cells (Fig. 6B). IL-28A showed higher antiproliferative properties than IL-29 at same concentrations, reducing proliferation up to 45% (Fig. 6B). This reduction in cell proliferation was significant for all cytokine concentrations used ($P < 0.005$ for IL-28A and $P < 0.05$ for IL-29). The antiproliferative effect correlated highly between the two IFN-λs ($r = 0.990$). Similar results were found for HT-29 cells (data not shown).

IFNs induce STAT1 phosphorylation. Previous studies in other cell systems reported activation of STAT signaling by

![Image](http://ajpgi.physiology.org/)

Fig. 2. IFN-λs activate ERK MAPKs in IECs. Activation and expression of phospho (p)-ERK1/2 was assessed by immunoblotting. A: p-ERK1/2 activation after stimulation with IL-28A (100 ng/ml). IL-28A-induced ERK activation is MEK-1 dependent. Pretreatment with the MEK-1 inhibitor PD-98059 (10 μmol/l 1 h before IL-28A stimulation) resulted in decreased ERK activation. B: similar results were obtained for stimulation with IL-29. The observed ERK1/2 activation was also dependent on MEK-1. One representative experiment ($n = 3$) is shown.

![Image](http://ajpgi.physiology.org/)

Fig. 3. IFN-λs activate stress-activated protein kinase (SAPK)/Jun NH2-terminal kinase (JNK) in IECs. Stimulation of HT-29 cells with IL-28A (100 ng/ml; A) or IL-29 (100 ng/ml; B) resulted in increased phosphorylation of SAPK/JNK. Pretreatment with the SAPK/JNK inhibitor SP-600125 (20 μmol/l) decreased SAPK/JNK activation. One representative experiment ($n = 3$) is shown.
IFN-αs (23, 32). Therefore, we investigated the influence of IL-28A and IL-29 on phosphorylation levels of STAT1 and STAT3 in HT-29 cells. As demonstrated in Fig. 7, both cytokines activated STAT1 but had, compared with IFN-α, no or only a minimal effect on STAT3 phosphorylation.

IFN-αs upregulate SOCS-3 mRNA expression. Activation of STAT proteins has been linked to increased SOCS-3 promoter activity and increased SOCS-3 gene transcription (2, 3, 33). Therefore, we analyzed whether IFN-αs influence STAT-dependent SOCS-3 mRNA expression levels in IEC. In these experiments, IL-28A and IL-29 (100 ng/ml each) clearly upregulated SOCS-3 mRNA expression in HCT116 cells up to four- or threefold, respectively (Fig. 8).

IFN-αs upregulate mRNA expression of the antiviral proteins 2’-5’-OAS and MxA. STAT signaling has not only been implicated in the regulation of SOCS-3 expression. Antiviral properties mediated by type I and type II interferons depend
also on STAT signaling (33). Therefore, we investigated the influence of IFN-λs on the transcriptional regulation of three antiviral proteins (PKR, 2',5'-OAS, and MxA) in HCT116 cells. mRNA expression of 2',5'-OAS and MxA was upregulated by IL-28A (2- and 3-fold increase, respectively) and IL-29 (11- and 5-fold increase, respectively), whereas mRNA levels of PKR remained unchanged (Fig. 9).

IFN-λs decrease the number of HCMV, immediate-early 1 protein, protein-positive, cells after infection in vitro. To investigate whether the activation of the antiviral proteins 2',5'-OAS and MxA results in antiviral activity in vitro, we analyzed the effect of IL-28A and IL-29 on the HCMV infection rate in HCT116 cells. In these experiments, both IL-28A and IL-29 decreased the number of cells positive for HCMV, immediate-early 1, protein as analyzed by immunostaining with an antibody against HCMV immediate-early protein (Fig. 10, A and B). This effect was highly significant (P < 0.001) for both cytokines at concentrations of 10 and 100 ng/ml (Fig. 10C) and could not be explained by the antiproliferative properties of these cytokines alone, which were considerably lower than their antiviral response as determined by measuring the protein concentration of IL-28A- and IL-29-stimulated HCT116 cells using the Bradford assay in parallel experiments (Fig. 10D).

IL-28A mRNA expression is upregulated after MCMV infection in vivo. Having demonstrated that IFN-λs mediate antiviral activity in vitro, we next analyzed their mRNA expression after viral infection in vivo using a murine model of MCMV infection. C57/BL6 mice were infected with 10^6 plaque-forming units of MCMV of the Smith strain (30). Forty-five hours after infection, IL-28A mRNA levels were clearly upregulated 3.8-fold in the colon of infected mice compared with noninfected mice.

DISCUSSION

IL-28A and IL-29 belong to a group of recently discovered IL-10-related cytokines. Five of these cytokines (IL-22, IL-26, IL-29, IL-28A, and IL-28B) use a common second chain, IL-10R2, to assemble their active receptor complexes. Thus IL-10R2 is a shared component in at least four distinct class II cytokine-receptor complexes (IL-10 receptor: IL-10R1; IL-22 receptor: IL-22R1; IL-26 receptor: IL-20R1; and IFN-λ recep-

Fig. 7. IFN-λs induce signal transducer and activator of transcription (STAT)1 phosphorylation in IECs. Upon stimulation of HT-29 cells with IL-28A (A) or IL-29 (B), STAT1 protein is phosphorylated, whereas STAT3 phosphorylation is not activated. Similar results were obtained in HCT116 cells (data not shown). IFN-α served as a positive control for STAT3 activation.

Fig. 8. IFN-λs upregulate suppressor of cytokine signaling (SOCS)-3 mRNA expression. Stimulation of HCT116 cells with 100 ng/ml IL-28A (A) or IL-29 (B) upregulated SOCS-3 mRNA expression up to 4- and 3-fold, respectively.
IL-28R, also synonymously named IFN-λ1). Although IL-10R2 is broadly expressed on a wide variety of tissues, only a subset of these tissues expresses the ligand-binding R1 chains.

Here, we demonstrated that the IFN-λR complex is expressed in IECs. Moreover, IL-28R is functional in IECs. After stimulation with IL-28A and IL-29, MAPKs, Akt, and STAT1 proteins are activated. In addition, we demonstrated that IL-28R mediates antiviral properties similar to other class I cytokine receptors. Specifically, stimulation of IECs with IL-28A and IL-29 resulted in increased mRNA expression of the antiviral proteins 2′,5′-OAS and MxA.

Having shown that IFN-λs activate the gene transcription of antiviral proteins, we next investigated the effect of these cytokines on the infection rate of HCMV in IECs. HCMV is an ubiquitous virus that is a major cause of morbidity and mortality in immunocompromised individuals (15). For example, during severe HCMV disease in AIDS patients, the gastrointestinal tract is frequently involved and is the second main site of infection, after the retina. Although any location within the gut may be affected, gastrointestinal HCMV disease typically produces mucosal erosion or ulceration with inflammation, tissue necrosis, and vascular injury resembling features of inflammatory bowel disease (19). HCMV infects a broad spectrum of gastrointestinal cells, notably, endothelial cells, smooth muscle cells, macrophages, and fibroblasts, but also IECs of the mucosa. Infection of the endothelial cells can explain the ischemic mucosal injury with concomitant necrosis. However, epithelial cells are frequently infected, suggesting that they may also be involved in the pathological process (8).

Antiviral therapy against HCMV relies on several effective drugs, such as ganciclovir, foscarnet, and cidofovir. Because of intense immunosuppression and frequent viral reactivations, HCMV disease tends to have a relentlessly progressive course in immunocompromised individuals such as AIDS patients (22). Therefore, patients with sight- or life-threatening manifestations are typically maintained on low-dose antiviral therapy indefinitely (21). However, the virus frequently develops resistance to antiviral agents, causing relapse of clinical symptoms (20, 34). Here, we demonstrated that IL-28A and IL-29 significantly inhibit the HCMV infection rate of IECs. Hence, these cytokines may be of interest as therapeutic agents, particularly in patients with serious side effects or drug resistance to current antiviral agents. This is not only supported by our in vitro results. Here, we also demonstrated for the first time in a murine in vivo study that colonic IL-28A mRNA expression is increased during CMV infection.

IL-28A and IL-29 are members of the IL-10-like cytokine family, sharing the IL-10R2 subunit for signal transduction. Interestingly, a viral IL-10 homolog encoded by an open reading frame (UL111a) within the human HCMV genome, designated HCMVIL-10, has been recently identified (24). HCMVIL-10 can bind to human IL-10R and can compete with human IL-10 for binding sites (24). HCMVIL-10 requires both subunits of the IL-10R complex to induce signal transduction events and biological activities (24). It has been demonstrated that HCMVIL-10 is expressed in HCMV-infected cells (24).

In addition to its antiviral functions, IFN-λs demonstrated antiproliferative properties. Because HT-29 and HCT116 cells, the IEC lines used for these experiments, are colorectal cancer-derived cells, it can be hypothesized that this IFN-λ-mediated function may be used for therapeutic purposes. Similarly, type I IFNs have been used as anticancer therapy (29). In particular, IFN-α, which shows similar to IFN-λs antiviral and antiproliferative properties, is commonly used as primary treatment for chronic hepatitis C virus infection and other clinical indications, including hepatitis B, melanoma, hairy cell leukemia, and non-Hodgkin’s lymphoma, suggesting that IFN-λs may be effective in these conditions too. Recently, we demonstrated that IFN-λs also upregulate the antiviral proteins MxA and 2′,5′-OAS in liver cells (7), further illustrating their therapeutic potential against hepatitis B and C, which is also supported by new in vitro findings (31). However, further animal and in vivo
studies are necessary to evaluate if the IFN-λ mediated anti-proliferative and antiviral functions may be used for human therapy. However, in contrast to their antiproliferative effects, no effect on IEC apoptosis could be demonstrated for IL-28A and IL-29 in our study.

IFN-λs activate STAT1 and STAT2, which can combine with another cytosolic protein, IRF-9 (p48), to form the ISGF3 transcription factor complex. The ability of IFN-λs to activate ISGF3 formation is reminiscent of signaling by type I IFNs and suggests that IFN-λs are evolutionary related to type I IFNs. It has been demonstrated that IL-29-induced STAT2 tyrosine phosphorylation is mediated through Tyr343 and Tyr517 of the receptor, which showed some similarities with tyrosines from type I IFN receptors involved in STAT2 activation (12). These two tyrosines were also responsible for antiviral and antiproliferative activities of IL-29 (12). Despite functional similarities between IFN-λs and IFN-α, we demonstrated differences in the signaling of these two cytokines. Whereas IFN-α is a strong activator of STAT1 and STAT3, IFN-λs only activate STAT1 phosphorylation. Moreover, IFN-α activates the antiviral proteins PKR (25), MxA, and 2′,5′-OAS (33), whereas we demonstrated that IFN-λs increase only mRNA expression of MxA and 2′,5′-OAS but not of PKR. Interestingly, similar differences were observed between IFN-β and IFN-γ. In the presence of IFN-β, but not IFN-γ, PKR expression and its phosphorylation were increased (10, 18).

In conclusion, we demonstrated that IECs express functional receptors for IFN-λs, which activate STAT and MAPK signaling, mediating antiviral and antiproliferative signals in IECs. Moreover, IL-28A mRNA expression is increased in mCMV infection in vivo. At present, the potential of IFN-λs in clinical applications is unknown, but the results of this and other studies (7, 23, 32) indicate that these cytokines should be further investigated clinically for similar applications as already proven for type I IFNs such as viral infections and malignancies. In addition, it is expected that phenotypic analysis of gene knockout mice will provide more details on the physiological roles of these novel cytokines.

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