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Antibody-based immunotherapy of aciclovir resistant ocular herpes simplex virus infections

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ABSTRACT

The increasing incidence of aciclovir- (ACV) resistant strains in patients with ocular herpes simplex virus (HSV) infections is a major health problem in industrialized countries. In the present study, the humanized monoclonal antibody (mAb) hu2c targeting the HSV-1/2 glycoprotein B was examined for its efficacy towards ACV-resistant infections of the eye in the mouse model of acute retinal necrosis (ARN). BALB/c mice were infected by microinjection of an ACV-resistant clinical isolate into the anterior eye chamber to induce ARN and systemically treated with mAb hu2c at 24 h prior (pre-exposure prophylaxis) or at 24, 40, and 56 h after infection (post-exposure immunotherapy). Mock treated controls and ACV-treated mice showed pronounced retinal damage. Mice treated with mAb hu2c were almost completely protected from developing ARN. In conclusion, mAb hu2c may become a reliable therapeutic option for drug/ACV-resistant ocular HSV infections in humans in order to prevent blindness.

1. Introduction

Herpes simplex viruses (HSV) are the leading infectious cause of blindness in industrialized countries (Farooq and Shukla, 2012) and a serious health problem in developing countries (Pramod et al., 2000). After acquisition of HSV via the orofacial route, the virus persists lifelong in the trigeminal ganglia (TGs) (Roizman and Whitley, 2013). Frequent reactivations of latent HSV are common and may be associated with ocular manifestations in the cornea or retina, leading to visual impairment and blindness. Infections of the cornea lead to the development of the herpetic stromal keratitis (HSK) (Burrel et al., 2013), while manifestations in the retina may result in acute retinal necrosis (ARN) (Lau et al., 2007). HSK is associated with severe immune-mediated inflammation of the cornea that may finally lead to opacity of the cornea and blindness. Worldwide, roughly 1.5 million HSK cases are reported (Farooq and Shukla, 2012). ARN is characterized by occlusive retinal vasculitis, prominent inflammation of the anterior segment and localized retinal necrosis that is often followed by optic neuritis and irreversible retinal detachment (Duker and Blumenkranz, 1991). Moreover, bilateral involvement of ARN (BARN) may occur within the first weeks of disease (Jeon et al.,; Palay et al., 1991). Further complications may be optic nerve head atrophy, macular edema, vessel occlusion, ocular hypotony, blindness in the diseased eye(s), glaucoma and encephalitis (Muthiah et al., 2007; Winterhalter et al.,). HSK and ARN can be caused by both HSV subtypes HSV-1 and HSV-2 (Grose, 2012; Lau et al., 2007).

Aciclovir has been used to treat ocular HSV manifestations for more than two decades (Blumenkranz et al., 1986). The current antiviral treatment for HSK and ARN consists of intravenous or oral therapy with aciclovir or newer antivirals including famciclovir (prodrug for

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penciclovir) or valaciclovir (prodrug for aciclovir) that have a better bioavailability in the central nervous system than aciclovir (Tibbetts et al., 2010). Furthermore, the current therapy for ARN often includes intraocular injection of antivirals. The antiviral treatment of HSK is supported by corticosteroids to suppress ocular inflammation (Knickelbein et al., 2009). Beside, prophylactic panretinal argon laser photocoagulations are performed in many cases of ARN to prevent retinal detachment (Park and Pavesio, 2008). However, the use of corticosteroids may cause unwanted side effects such as the development of glaucoma and cataract (Carnahan and Goldstein, 2000; Gaudio, 2004). Moreover, long-term antiviral treatment may lead to the development of resistance towards aciclovir (Duan et al., 2008; Toriyama et al., 2014; van Velzen et al., 2013a). The frequency of ACV-resistant strains among patients with ocular HSV manifestations was described by van Velzen et al. to be 26% (van Velzen et al., 2013b). ACV or multidrug-resistant strains could be isolated from patients suffering from HSK (Toriyama et al., 2014; van Velzen et al., 2013b) or ARN (Dokey et al., 2014; Patel et al., 2010; Tran et al., 2005). Strains resistant to aciclovir were described to be cross-resistant to penciclovir (Bacon et al., 2003). Alternative antivirals such as cidofovir or foscarnet are available but may be associated with severe side effects (Broekema and Dikkers, 2008; Jacobson, 1992). Clearly, a novel effective and well tolerable antiviral therapy is needed for the treatment and prevention of ACV-resistant ocular HSV infections. We have recently reported the development of a humanized, highly neutralizing monoclonal antibody (mAb hu2c) for the treatment of drug-resistant HSV infections (Krawczyk et al., 2013, 2011). Systemic mAb hu2c immunotherapy was capable of protecting highly immunodeficient NOD/SCID mice from lethal HSV infection. Furthermore, mAb hu2c potently protected mice from the development of HSK after corneal infection with HSV-1 KOS (Krawczyk et al., 2015). Based on these prior data, we hypothesized that mAb hu2c should be particularly suitable for the otherwise hard to treat ACV-resistant infections of the eye. The aim of the present study was to clarify whether mAb hu2c is actually capable of preventing the development of ARN induced by the infection with an ACV-resistant strain. We used three clinical HSV-1 isolates resistant to ACV that were isolated from patients with severe HSK. We characterized these isolates by detailed pheno- and genotypic resistance analysis and identified deletions and mutations associated with reduced susceptibility towards aciclovir. Subsequently, we identified one isolate that was reliable to induce the ARN in BALB/c mice according to the previously described ARN mouse model (Whittum et al., 1984). Similar to the emergence of ocular infection upon HSV reactivation in humans, the establishment of disease in this model requires effective viral spread from sensory neurons to the periphery (Whittum et al., 1984). After microinjection of HSV-1 strain KOS into the anterior chamber of the left eye, the virus replicates in the anterior segment of this eye. The virus then spreads from the initially infected eye via parasympathetic neurons to the ipsilateral ciliary ganglion and reaches the contralateral optic nerve and retina at days 6–7 post infection resulting in the development of ARN (Atherton, 2001; Bosem et al., 1990; Cathcart et al., 2009; Vann and Atherton, 1991; Whittum et al., 1984). Finally, we examined the efficacy of an antibody-based immunotherapy with mAb hu2c towards ACV-resistant ocular HSV infections in the mouse model of ARN.

2. Material and methods

2.1. Viruses and cells

Three ACV-resistant HSV-1 isolates (HSV-1 ACVr 1, HSV-1 ACVr 2 and HSV-1 ACVr 3) from patients with severe ocular infections were propagated on Vero cells (American Type Culture Collection, ATCC, CCL81, Rockville, MD) and stored at −80 °C. HSV-1 ACVr 1 was described in van Velzen et al. (2013b) JID; PMID: 23901090 among a number of isolates derived from patients unsuccessfully treated with ACV. HSV-1 ACVr 2 was described as isolate R6 in Duan et al. (2008) JID; PMID: 18627246 and HSV-1 ACVr 3 as isolate R9 A in Duan et al. (2009) JID; PMID: 19795980. Vero cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Life Technologies Gibco, Darmstadt, Germany) containing 10% (v/v) FCS.

2.2. Phenotypic and genotypic drug-resistance testing

The susceptibility of HSV-1 strains to the antiviral drugs aciclovir (ACV), foscarnet (FOS) and cidofovir (CDV) has been analyzed by a standard plaque reduction assay on Vero cells as previously described (Kruppenbacher et al., 1994; Pavic et al., 1997). The median inhibitory concentration (IC_{50}) was defined as the drug concentration that reduced viral loads by 50% when compared to untreated control infected cells. Genotypic resistance testing was performed by sequence analysis of the thymidine kinase (TK) gene UL23 and the polymerase gene UL30. The identification of mutations associated with drug resistance was done using the online-tool “Mutation Analyzer” at the University of Ulm, Germany (Analyzer; Chevillotte et al., 2010). All sequences were deposited in NCBI GenBank under the accession numbers MF093721 to MF093726.

2.3. Antibodies

Monoclonal antibodies mAb 2c and mAb hu2c were produced and purified as described before (Krawczyk et al., 2013, 2011). Purity was confirmed by FPLC ≥ 95%. Concentration was measured with a NanoDrop 2000 spectrometer (Thermo Scientific, Wilmington, DE, USA).

2.4. Animal experiments

Female BALB/c mice, 7–8 weeks of age, were purchased from Charles River Laboratories (Charles River Laboratories, Sulzfeld, Germany) and maintained under pathogen free conditions according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The in vivo experiments were conducted according to the German legal requirements with the approval of the University Hospital Essen's animal facility.

2.5. Murine model of HSV retinitis

Female BALB/c mice (Charles River Laboratories, Sulzfeld, Germany) were infected with ACV-resistant HSV-1 isolates (HSV-1 ACVr 1, HSV-1 ACVr 2 and HSV-1 ACVr 3) to induce HSV retinitis as previously described (Pepose and Whittum-Hudson, 1987). Briefly, mice were anesthetized by intraperitoneal injection of ketamine hydrochloride (2 mg) and mepivacaine hydrochloride (400 ng). Subsequently, mice were infected with various concentrations of the ACV-resistant HSV-1 isolates by microinjection of 2.5 µl of the virus preparation into the anterior eye chamber of the right eye. Intraocular injections were performed with a 30-gauge needle attached to a 100 µl microsyringe (Hamilton, Reno, NV). The incidence of ocular disease was determined by light microscopy. Further experiments were performed with the clinical isolate HSV-1 ACVr 1. Mice were infected with 5×10^3 PFU HSV-1 ACVr 1 as described above and systemically treated with the parental murine (mAb 2c) or the humanized (mAb hu2c) monoclonal antibody. The antibodies were applied intravenously by injection of 300 µg mAb 2c or mAb hu2c either 24 h prior to infection for pre-exposure prophylaxis (P), or 24, 40, and 56 h after infection for post-exposure immunotherapy (PEP). Control mice were either mock (PBS) treated or received ACV standard therapy at 10 mg/kg body weight or high-dose therapy at 50 mg/kg body weight three times a day (every 8 h) by intraperitoneal injection, starting at 24 h post infection. Mice were sacrificed 12 days post infection. The incidence of ocular disease was determined by light microscopy and hematoxylin-eosin staining of the eye sections. Additionally, viral loads in the contralateral eyes were measured on day 8 post infection with a standard plaque

assay (N = 6 in each group).

2.6. Viral loads in the eye

Contralateral eyes of representative mice (n = 6 each group) were enucleated on day 8 p.i. and immediately snap frozen. Subsequently, the eyes were homogenized and serial 1:10 dilutions of the homogenate were incubated on Vero cell monolayers for 1 h at 37 °C. The inoculation medium was then removed and the monolayers were covered with RPMI-agarose medium and incubated for 3 days. To determine the viral load, the cells were fixed and stained with 2% crystal violet. Plaques were counted, and the number of PFU/ml was calculated based on the dilution factor as previously described (Bauer et al., 2000).

2.7. Virus reactivation from trigeminal ganglia

The ipsi- and contralateral trigeminal ganglia (TGs) were explanted on day 12 after infection and examined for HSV reactivation by co-cultivation with Vero cells as previously described (Krawczyk et al., 2015).

2.8. Histology

The incidence of retinal damage was investigated by light microscopic examination of hematoxylin-eosin stained eye sections. For this purpose, eyes were harvested on day 12 post infection, fixed (64% isopropanol, 3.7% formaldehyde, 2.5% acetic acid), dehydrated with isopropanol, and embedded in paraffin. Five-micrometer sections were then stained and analyzed as previously described (Hennig et al., 2012).

2.9. Statistical analysis

Data were analyzed using GraphPadPrism 5 (GraphPadPrism Software, La Jolla, CA, USA).

The differences between the number of mice showing signs of ARN and healthy mice (Fig. 2) or of latently infected trigeminal ganglions and the number of trigeminal ganglions exhibiting reactivation (Fig. 4) were examined by Fisher's exact test. Statistical analysis of the viral loads in the eyes (Fig. 3) was performed with nonparametric ANOVA (Kruskal–Wallis) and post hoc Dunn's multiple-comparisons test. Comparisons were considered significant at $^{*}P < 0.05$; $^{**}P < 0.01$; and $^{***}P < 0.001$.

3. Results

3.1. Pheno- and genotypic characterization of ACV resistant HSV-1 clinical isolates

Three HSV-1 strains isolated from patients with severe HSK resistant to antiviral treatment with ACV were used for the study. The isolates were characterized as ACV-resistant with a phenotypic cell culture assay (Fig. 1). The threshold for ACV-resistance was described with 2 µg/ml (equal to 8.89 µM) in prior studies (Bacon et al., 2003; Safrin et al., 1994; Sauerbrei and Bohn, 2014). We therefore considered all of the three isolates as ACV resistant. ACV resistance could be further confirmed by a genotypic characterization. The HSV-1 strain ACVr 2 exhibited a deletion of isoleucine at position 194 (I194del), and ACVr 3 the mutation M128L within the thymidine kinase (TK) gene. Both mutations had been described as resistance-associated mutations by Duan et al. (2009). The sequence analysis of the isolate ACVr 1 revealed a substitution of threonine to arginine at amino acid position 66 (T66R) in a conserved region of the TK-gene. This mutation has not yet been described, but former studies reported that mutations within the conserved region of the TK are mainly associated with drug resistance (Sauerbrei et al., 2016). The sequences of the conformational epitope recognized by mAbs 2c and hu2c were conserved among the isolates (Fig. 1). All three isolates were neutralized with the same efficacy by mAb 2c and hu2c (7.8 nM) as non-resistant HSV (data not shown).

3.2. Induction of ARN with ACV-resistant HSV-1 clinical isolates in BALB/c mice

Strains of HSV-1 have been reported to vary in their pathogenesis and neuroinvasiveness in mice (Wang et al., 2013). Therefore, we initially tested the three ACV-resistant HSV-1 clinical isolates at different viral loads ranging from 1×10^3 PFU/2.5 µl to 1×10^5 PFU/2.5 µl for their capacity to induce ARN in BALB/c mice. The HSV-1 ACV-resistant strain 1 (van Velzen et al., 2013b) proved to be appropriate to induce ARN in mice when used at 5×10^3 PFU/inoculation. Besides this, the virus induced a pronounced inflammation of the anterior segment of the contralateral eye. The histology of the contralateral eye showed a strong retinal necrosis, comparable to the histological picture following infection with the widely used HSV-1 KOS strain. The HSV-1 ACVr isolate 3 revealed to be highly virulent and rapidly killed the mice between day 5 and 8 before ARN was established, while HSV-1 ACVr isolate 2 was low virulent and did not induce ARN (Fig. 1). We therefore selected the HSV-1 ACVr 1 isolate to conduct the following experiments.

3.3. Prevention of HSV-1 ACVr-induced infection of the retina by mAbs 2c and hu2c

To investigate the efficiency of mAb 2c and mAb hu2c in the prevention of ARN, mice were infected with 5×10^3 PFU of the HSV-1 ACVr 1 isolate. The antibodies were systemically applied 24 h before (pre-exposure prophylaxis (P)) or 24 h, 40 h and 56 h after infection (post-exposure immunotherapy (PEP)). On day 12 post infection, the contralateral eyes were examined for HSV-1 specific pathology in the anterior part of the eye. In untreated control mice, 90% (9/10) of the animals developed acute inflammation of the anterior eye segment with corneal edema, iris vessel dilatation, mydriasis and posterior synechiae, and fibrin deposits on the lens (Fig. 2). Pathological changes of the

A

HSV-1 isolate	**ACV** IC_{50} [µmol/L]	FOS IC_{50} [µmol/L]	CDV IC_{50} [µmol/L]	Resistance associated mutation in TK	Lethal in mice within 12 days	Induces ARN
HSV-1 ACVr 1	**10.00**	51.83	1.27	T66R*	**no**	**yes**
HSV-1 ACVr 2	**79.5**	102.43	2.33	I194del PMID: 19795980	no	no
HSV-1 ACVr 3	**31.93**	108.43	4.87	M128L PMID: 19795980	yes	no

B

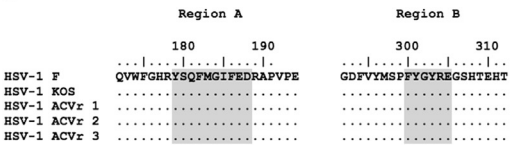

Fig. 1. Resistance profile of three clinical HSV-1 isolates collected from patients with HSK. **(A)** Three clinical isolates (HSV-1 ACVr 1, HSV-1 ACVr 2 and HSV-1 ACVr 3) resistant towards ACV were titrated for their sensitivity to the antiviral drugs ACV, foscarnet (FOS) and cidofovir (CDV) and tested for their pathogenicity in the ARN mouse model. Resistance mutations within the viral thymidine kinase gene were determined by sequencing and database analysis with HSV reference sequences. The median inhibitory concentration (IC_{50}) was defined as the drug concentration that reduced viral loads by 50% when compared to untreated control infected cells. The cutoff value for aciclovir resistance was $IC_{50} > 4$ µmol/L, for foscarnet resistance $IC_{50} > 300$ µmol/L and for cidofovir $IC_{50} > 10$ µmol/L. **(B)** Conservation of the mAb hu2c gB epitope sequences was examined by sequencing of the gB-gene and is indicated at amino acid level. Motifs A ($_{179}$YSQFMGIFED$_{188}$) and B ($_{300}$FYGYRE$_{305}$) forming the discontinuous epitope recognized by mAb hu2c are indicated in gray.

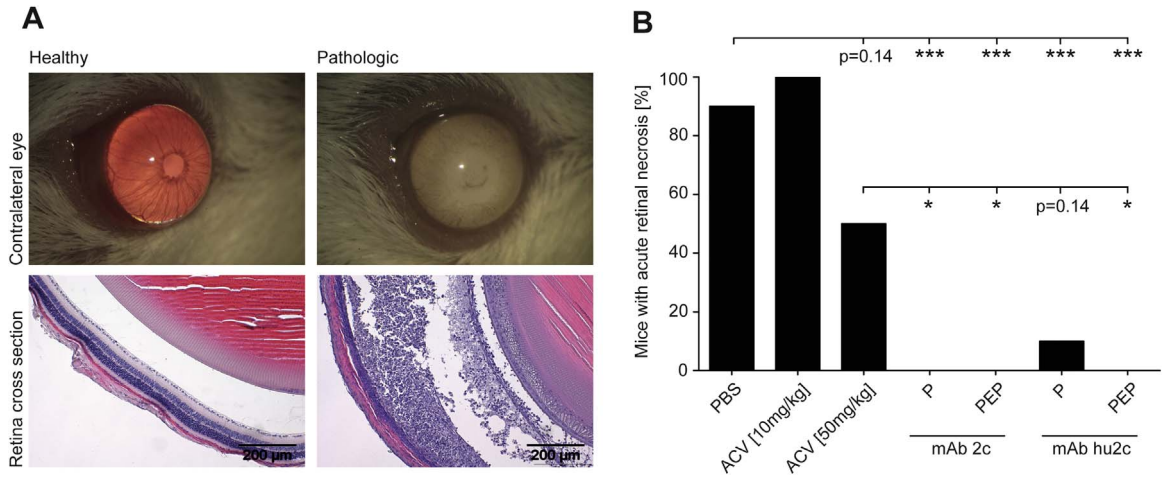

Fig. 2. Efficacy of mAbs 2c and hu2c in the prevention of ACV-resistant HSV-1 infection of the retina. **(A)** The contralateral eyes were examined at day 12 post infection for retinal disease by light microscopy and histological staining and classified as pathologic or healthy. Healthy eyes were characterized by a normal clinical appearance and intact retina, whereas pathologic eyes were impaired and showed extensive damage of the retinal tissue. **(B)** Percentages of mice (n = 10) with pronounced retinal necrosis. The impacts of antibody treatment at 24 h before infection (P) or 24, 40 and 56 h after infection (PEP) were compared with mock (PBS) or high-dose ACV (50 mg/mg) treated mice and statistical significances were determined with the Fisher´s exact test. Comparisons were considered significant at *P < 0.05; **P < 0.01; and ***P < 0.001.

retina were proven by histological analysis and confirmed the clinical characteristics of a severe retinal necrosis. A similar clinical picture and a similar pronounced retinal necrosis was found in ACV-treated mice. In contrast, antibody treated mice were effectively protected from the development of ARN. Mice treated with the murine antibody 2c showed no signs of disease when applied before or after infection. A similar therapeutic effect was achieved with the humanized mAb hu2c. All but one of the prophylactically mAb hu2c treated mice were protected from severe ocular disease.

3.4. Effect of antibody treatment on the viral load in the contralateral eye

To investigate the effect of systemic antibody treatment with mAbs 2c or hu2c on the virus content in the eyes, six representative mice per group mice were sacrificed on day 8 post infection. The contralateral eyes were removed, shock-frozen and stored at −80 °C. To determine the viral load, the specimens were homogenized at 4 °C and analyzed by a standard plaque assay on Vero cells. As shown in Fig. 3, the contralateral eyes of the control mice contained high viral loads. ACV-treated mice showed slightly decreased viral loads in the eyes, but the difference to controls was not statistically significant. In contrast, there was no virus detectable in the contralateral eyes of mice treated with mAb 2c or mAb hu2c, irrespectively of whether the mice were treated prophylactically (P) or a post-exposure prophylaxis (PEP) was given (***P < 0.001) (Fig. 3).

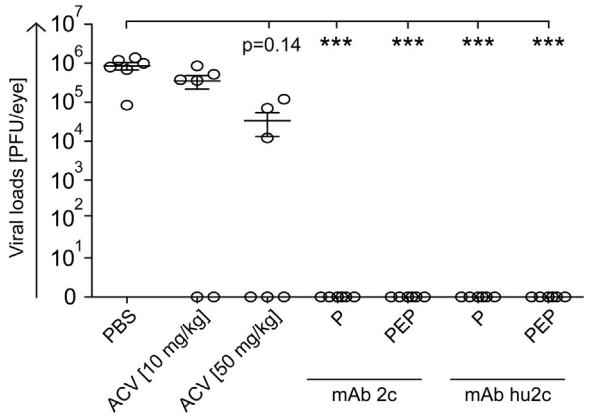

Fig. 3. Potent protection of the contralateral eyes against HSV-1 infection by systemically applied mAbs 2c or hu2c. Eyes were removed on day 8 post infection, homogenized and tested for viral loads using a standard plaque assay. The impacts of antibody treatment at 24 h before infection (P) or 24, 40 and 56 h after infection (PEP) were compared with mock (PBS) treated mice and statistical significances were determined with a nonparametric ANOVA (Kruskal–Wallis) and post hoc Dunn's multiple-comparisons test. Comparisons were considered significant at *P < 0.05; **P < 0.01; and ***P < 0.001.

3.5. Reactivation of ACV-resistant HSV-1 from trigeminal ganglia

Almost all antibody-treated mice were completely protected from the development of ARN, and there was no virus detectable in the contralateral eyes. Therefore, we hypothesized that P or PEP treatment with mAbs 2c or hu2c would be capable of inhibiting the cell-associated transmission of HSV-1 from the periphery to the trigeminal ganglia. To answer this question we isolated the TGs from the ipsilateral and the contralateral site on day 12 post infection. The TGs were then co-cultured with Vero cells for 3 weeks and daily checked for the development of cytopathic effects complying with virus reactivation. HSV-1 reactivation was found in the ipsilateral (10/10) and contralateral TGs (9/10) of the untreated control mice. After standard-dose ACV treatment (10 mg/kg), HSV reactivation was detected in 10/10 ipsilateral and 8/10 contralateral TGs (Fig. 4). A slightly improved effect could be observed in mice treated with high-dose ACV (50 mg/kg). HSV reactivation was observed in almost all ipsilateral TG (9/10) and 50% (5/10) of contralateral TGs. The most pronounced effects could be achieved when monoclonal antibodies were used. There was a lower number of HSV reactivating ipsilateral TGs of mAb 2c (P: 7/10; PEP: 8/10) and hu2c (P: 9/10, PEP: 9/10) treated mice. In contrast, no reactivation was observed in the contralateral TGs obtained from mAb 2c treated mice (P: 0/10; PEP: 0/10). Similarly, in mAb hu2c treated mice, reactivation was found only in one TGs (P: 0/10, PEP: 1/10). These results indicate that the cell-associated transmission of ACV-resistant HSV-1 from the site of primary infection (ipsilateral eye) to the contralateral TGs was successfully inhibited by mAbs 2c and hu2c.

4. Discussion

Frequent reactivations of herpes simplex virus (HSV) from the trigeminal ganglia may lead to the recurrent infection of the cornea or retina. Both manifestations may be associated with severe impairment of sight or blindness. The treatment of ocular HSV infections can be challenging, particularly due to the increasing emergence of ACV resistances in patients suffering from ARN or HSK (Dokey et al., 2014; Patel et al., 2010; Toriyama et al., 2014; Tran et al., 2005; van Velzen

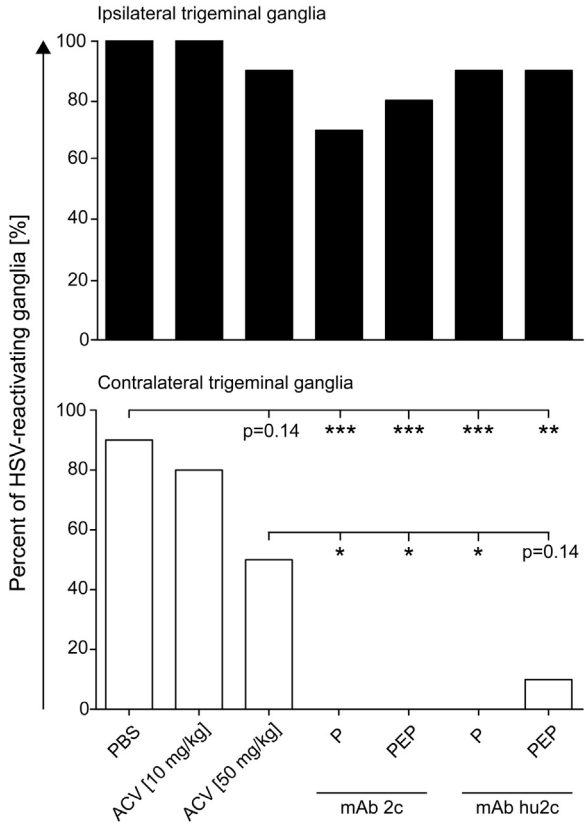

Fig. 4. Reactivation of ACV-resistant HSV-1 from trigeminal ganglia of mice systemically treated with mAbs 2c or hu2c. The percentages of reactivating ipsilateral or contralateral trigeminal ganglia from HSV-1 ACVr 1 infected mice (n = 10) are shown. Trigeminal ganglia were harvested on day 12 after infection and co-cultured with Vero cells for three weeks. The occurrence of a cytopathic effect typical for HSV was considered as reactivation. The differences in the total numbers of reactivating ganglia between the antibody treatment groups and the PBS or ACV (50 mg/kg) group were compared and statistical significances were determined with the Fisher´s exact test. Comparisons were considered significant at *P < 0.05; **P < 0.01; and ***P < 0.001.

et al., 2013b)

Due to the persistent need to improve the conventional therapy and to overcome drug resistant ocular infections, we investigated the antiviral efficiency of mAb hu2c toward an ACV-resistant clinical isolate in the mouse model of acute retinal necrosis. Monoclonal antibodies were described as a potent tool for the treatment of HSV infections (Atherton, 1992; Eis-Hubinger et al., 1993; Krawczyk et al., 2013, 2015; Metcalf et al., 1988, 1987).

We hypothesized that the HSV-1/2-gB specific, humanized monoclonal antibody mAb hu2c, developed by our group might be a good candidate for the prevention of ACV-resistant ocular infections. The epitope recognized by this antibody is highly conserved among a broad range of HSV1/2-isolates and essential for viral fitness (Daumer et al., 2011). The unique feature of this antibody is its ability to completely abort the cell-to-cell transmission of the virus, which makes it superior to polyclonal HSV-neutralizing human antibodies (Krawczyk et al., 2013, 2011). Cell-to-cell spread is used by HSV to escape from the host's immune response. This mechanism is usually unassailable by human antibodies and requires among others the viral glycoprotein gB (Cocchi et al., 2000; Sattentau, 2008a, 2008b). The superiority of the parental murine antibody to neutralizing human antibodies was demonstrated in a prior study in the murine genital herpes model (Eis-Hubinger et al., 1993). The assessment of ARN requires an effective anterograde-directed transport of HSV from the trigeminal ganglia to the retina that proceeds over the cell-associated cell-to-cell transmission of the virus (Al-Dujaili et al., 2011). The HSV-1 ACVr 1 isolate from a patient suffering from HSK used for the mouse study was characterized by phenotypic analysis as resistant toward aciclovir. The mutation found in the TK-gene (T66R) might be responsible for the ACV resistance (Sauerbrei et al., 2016). Additionally, we found a mutation in the polymerase gene (D672N), which also might be associated with resistance (Burrel et al., 2010; Sauerbrei et al., 2010). Further investigations are needed to clarify the exact impact of these mutations on ACV resistance. The recommended regime for the treatment of ARN in humans is intravenous application of acyclovir at 10 mg/kg every 8 hours per day (Tam et al., 2010). In infants with CNS disease suffering from herpes neonatorum doses of 40–60 mg/kg are used (Kimberlin et al., 2001). Accordingly, we applied aciclovir at the standard dosage (10 mg/kg) or as high-dose therapy (50 mg/kg) in our study. ACV completely failed to protect the mice from ARN at 10 mg/ml and was only 50% effective at 50 mg/ml. The data indicate that high-dose ACV therapy may improve the clinical outcome of infection resistant to standard treatment regimens (Kim et al., 2011). In contrast to ACV treatment, systemically applied mAbs 2c or hu2c effectively protected mice from ARN when applied at 24 h before or 24 h, 40 h and 56 h after infection. In this model, the viral transmission from the infected eye to the ipsilateral eye could not be inhibited by mAb 2c or mAb hu2c. The best protective effect was achieved when mAb 2c was applied at 24 h before infection, since there was no virus detectable in 3/10 ipsilateral ganglia. According to prior studies describing the ARN mouse model, the mice were observed for 12 days (Atherton, 1992). The average biological half-life is assumed with 22.4 days for total IgG antibodies (Thurmann et al., 1995). Hence, it is possible that there might be some persistent virus present after day 12 that might cause disease at later time points when the level of antibody already begins to wane.

With one exception (mAb hu2c PEP group), no virus could be reactivated from the contralateral ganglia of antibody treated mice. Consistent with our previous findings, our data indicate that the monoclonal antibodies not only neutralize the secreted virus but also abort the cell-associated viral transmission between cells and neurons (Krawczyk et al., 2015). Although HSV could be neither detected by cell culture reactivation assay nor by PCR amplification of HSV-DNA from the contralateral trigeminal ganglia (data not shown), further examinations are needed to clarify the underlying mechanism by which the antibody blocks the anterograde transmission of HSV. However, limiting the neuronal spread of the virus is crucial for the effective protection from ARN. Our findings are consistent with a prior study demonstrating that a monoclonal antibody (8D2) targeting a type-common epitope of glycoprotein D was effective in protecting from ARN (Atherton, 1992). Although the mode of action was not completely resolved, the authors hypothesized that this antibody may protect by blocking the viral spread through the central nervous system.

The murine ARN model used in this study mimics the anterograde-directed neuronal spread of HSV and the development of ocular infection in humans, but has some limitations. Most important, in contrast to some species like humans or rabbits, mice do not exhibit spontaneous reactivations of HSV (Gebhardt and Halford, 2005). Second, the course of infection differs between humans and mice. Humans are treated after the onset of symptoms. In contrast, HSV-relating symptoms are almost completely irreversible in mice. Therefore, potential antivirals are commonly applied up to 24 h post infection as post-exposure prophylaxis, after the establishment of infection but before the onset of symptoms (Kleymann et al., 2002; Palliser et al., 2006). Animal studies with ACV go back to the 1970's, when ACV was examined in experimental herpes simplex encephalitis in mice. The treatment was initiated 12 h post infection with a lethal HSV dose (Park et al., 1979). In humans, ACV was highly effective and became the golden standard for the treatment of HSV infections. The drug is commonly applied after the onset of infection or as prophylaxis in high-risk patients such as bone marrow transplants. In our study, the efficacy of mAb hu2c was compared to ACV, and we found that this antibody was highly effective in protecting mice from the development of ARN. We believe that this antibody may become a novel therapeutic option for ARN in humans,

and a good alternative to ACV. In humans, mAb hu2c may be used as prophylactic treatment in patients suffering from frequent reactivations of ocular herpes simplex virus, thereby preventing the damage of the eye and protecting from disease. Furthermore, mAb hu2c may be used for the treatment of florid ocular HSV infections to stop the progression of disease.

In conclusion, our results demonstrate the antiviral potency of the humanized mAb hu2c in the prevention of drug resistant HSV-infections of the eye. The antibody might be further evaluated for its clinical use in Phase I and II trials. We are highly confident that mAb hu2c will become a potent treatment option for patients with severe ocular diseases caused by ACV- and multi-resistant herpes simplex viruses, which are a major cause of blindness world wide.

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Conflict of interest

The authors have no financial or personal conflicts of interest.

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