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A human monoclonal antibody targeting SR-BI precludes hepatitis C virus infection and viral spread *in vitro* and *in vivo*

Philip Meuleman¹, Maria Teresa Catanese², Lieven Verhoye¹, Isabelle Desombere¹, Ali Farhoudi¹, Christopher T. Jones², Timothy Sheahan², Katarzyna Grzyb³, Riccardo Cortese³, Charles M Rice², Geert Leroux-Roels¹, and Alfredo Nicosia³

Philip Meuleman: philip.meuleman@ugent.be; Maria Teresa Catanese: mcatanese@mail.rockefeller.edu; Lieven Verhoye: lieven.verhoy@ugent.be; Isabelle Desombere: isabelle.desombere@ugent.be; Ali Farhoudi: ali.farhoudi@ugent.be; Christopher T. Jones: ctjones1@gmail.com; Timothy Sheahan: tsheahan@mail.rockefeller.edu; Katarzyna Grzyb: grzyb@ceinge.unina.it; Riccardo Cortese: cortese@ceinge.unina.it; Charles M Rice: ricec@mail.rockefeller.edu; Geert Leroux-Roels: geert.lerouxroels@ugent.be; Alfredo Nicosia: nicosia@ceinge.unina.it

¹Center for Vaccinology, Ghent University and Hospital, Gent, Belgium

²Center for the study of Hepatitis C, The Rockefeller University, New York, NY, USA

³CEINGE Biotechnologie Avanzate, Naples, Italy

Abstract

End-stage liver disease caused by chronic hepatitis C virus (HCV) infection is the leading indication for liver transplantation in the Western world. However, immediate re-infection of the grafted donor liver by circulating virus is inevitable and progresses much faster than the original disease. Standard antiviral therapy is not well tolerated and usually ineffective in liver transplant patients while anti-HCV immunotherapy is hampered by the extreme genetic diversity of the virus and its ability to spread via cell-cell contacts.

We have generated a human monoclonal antibody against SR-BI, mAb16-71, that can efficiently prevent infection of Huh-7.5 hepatoma cells and primary hepatocytes by cell-culture-derived HCV (HCVcc). Using an *Huh7.5* co-culture system we demonstrated that mAb16-71 interferes with direct cell-to-cell transmission of HCV. Finally we evaluated the *in vivo* efficacy of mAb16-71 in ‘human liver uPA-SCID mice’ (chimeric mice). A two-week anti-SR-BI therapy that was initiated one day before viral inoculation completely protected all chimeric mice from infection with serum-derived HCV of different genotypes. Moreover, a 9-day post-exposure therapy that was initiated 3 days after viral inoculation (when viremia was already observed in the animals) suppressed the rapid viral spread observed in untreated control animals. After cessation of anti-SR-BI-specific antibody therapy, a rise of the viral load was observed.

Conclusion—Using *in vitro* cell culture and human liver-chimeric mouse models, we show that a human monoclonal antibody targeting the HCV co-receptor SR-BI completely prevents infection and intrahepatic spread of multiple HCV genotypes. This strategy may be an efficacious way to prevent infection of allografts following liver transplantation in chronic HCV patients, and may even hold promise for the prevention of virus rebound during or following anti-viral therapy.

Keywords

liver transplantation; HCV; chimeric mice; prevention; immunotherapy

*Correspondence should be addressed to: Prof. Dr. Philip Meuleman, Center for Vaccinology - Ghent University, UZ Gent, Building A, 1st floor, De Pintelaan 185, B-9000 Gent, Belgium, Phone: +32 9 332 02 05, Fax: +32 9 332 63 11, philip.meuleman@ugent.be.

Introduction

With approximately 3% of the world's population infected with the hepatitis C virus (HCV), end-stage liver disease caused by this infection is currently the most common indication for liver transplantation (1). However, the donor liver almost inevitably becomes infected by circulating virus, and disease progression is accelerated in immune suppressed transplant patients (2). Less than 30% of liver transplant patients treated with pegylated interferon therapy with or without ribavirin will achieve a sustained virological response and this combination therapy is often not well tolerated (3–5). Therefore new strategies to prevent graft re-infection are urgently needed. In the coming years, new direct antiviral compounds will considerably improve therapy outcome in patients without severe liver disease (6–8), but the side effects and potential drug-drug interactions associated with triple therapy may severely complicate their use in liver transplant patients with end-stage liver disease (9–12). Because of the extreme genetic diversity of HCV and its ability to spread via cell-cell contacts, successful immunotherapy with polyclonal or monoclonal HCV-specific antibodies may be difficult to achieve (13–17). In contrast, viral (co-)receptors are genetically conserved and may represent better therapeutic targets. HCV entry is a multistep process in which different putative attachment factors and viral receptors are involved (reviewed in (18–20)). While heparan sulfate proteoglycans and the LDL-receptor are considered to be primary attachment factors; scavenger receptor class B type I (SR-BI/Cla1) (21–27), CD81 (28), claudin-1 (29) and occludin (30) seem to be actively involved in the entry process. After initial attachment, the viral particle directly and/or indirectly interacts with SR-BI, which together with CD81 triggers downstream events involving both claudin-1 and occludin.

We have previously shown that blockade of the tetraspanin CD81 can prevent *in vivo* infection by different HCV strains. However, the beneficial effect of this approach was virtually abolished when CD81 antibody was administered six hours after the virus injection (31), a likely consequence of the ability of HCV to efficiently disseminate via cell-cell contacts in a CD81-independent manner (32, 33). Although the role of CD81 in direct cell-to-cell transmission is still a matter of debate, claudin-1, occludin and especially SR-BI seem to play a prominent role in this process (34). We have generated a human IgG4 monoclonal antibody (mAb16-71) that targets SR-BI. Using the HCV cell culture system (HCVcc) (35–37), primary human hepatocyte cultures that faithfully recapitulate the polarized nature of hepatocytes *in vivo* (38, 39), and a human liver-chimeric mouse model (40–42), we show here that mAb16-71 prevents infection and viral spread of multiple HCV genotypes. Thus, this antibody is an attractive candidate molecule for preventing infection of allografts and recurrent chronic hepatitis following liver transplantation in chronic HCV patients, and for preventing the emergence of escape mutants and virus rebound during or following anti-viral therapy.

Materials and Methods

(A detailed description of the methods used can be found in the online supplement.)

Cells and antibodies

Huh-7.5 cells were maintained at 37°C, 5% CO₂ in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen) containing 10% fetal bovine serum (FBS) and 0.1 mM non-essential amino acids (NEAA). EGFP-IPS/CD81^{neg} cells have been previously described (43) and were grown in complete media containing 6 µg/ml blasticidin.

Primary adult and fetal cell cultures were established as described before (38, 39). Jc1 and J6/JFH-1 Clone 2 (44) HCVcc stocks were produced by electroporation of *in vitro* transcribed RNA into Huh-7.5 cells, as described previously (35).

Mouse experiments

Chimeric mice were produced as previously described (40). All animals used in this study received hepatocytes from a single donor and the study protocol was approved by the animal ethics committee of the Faculty of Medicine and Health Sciences of the Ghent University. The effectiveness of the different antibodies was evaluated in a prophylactic and post-exposure setting. For the prophylactic treatment, chimeric mice received a 2-week antibody therapy consisting of 5 intraperitoneal injections (day -1, 1, 5, 8 and 12), each containing 400 µg mAb16-71. One day after the first antibody injection (day 0), all mice were inoculated with a viral dose that was previously shown to infect all challenged animals (MID₁₀₀) of the following HCV strains: mH77C (genotype 1a; 10⁴ IU/mouse), mED43 (genotype 4a; 10⁴ IU/mouse) or mHK6a (genotype 6a; 10⁵ IU/mouse) (16, 17). The challenge viruses mH77C, mED43 and mHK6a were produced by infecting different naïve chimeric mice (hence the prefix “m”) with a pool of acute phase plasma derived from chimpanzees infected with H77C, ED43 and HK6a, respectively (45). For the post-exposure treatment, chimeric mice were first infected with mH77C virus, while treatment with mAb16-71 or CD81 antibody (clone JS81, BD Biosciences) was initiated three days later. Treated animals received 5 intraperitoneal antibody injections at day 3, 5, 7, 10 and 12; each containing 400 µg antibody.

Statistics

To analyze whether the difference between treatment groups was statistically significant, the data obtained was analyzed using the unpaired nonparametric two-tailed Mann-Whitney test. Data was analyzed using GraphPad InStat v3.0b (GraphPad Software Inc., La Jolla, CA).

Results

Anti-SR-BI antibody therapy prevents HCV infection *in vitro*

To investigate whether SR-BI blockade could prevent HCV infection we developed a human IgG4 monoclonal antibody that targets SR-BI, designated mAb16-71. The amino acid sequence of mAb16-71 is identical to that of antibody C167 which we produced earlier (24), but the gene sequence was codon-optimized to achieve higher and more efficient production. The antiviral efficacy of mAb16-71 was first evaluated in the HCVcc system. One day after seeding in a 96-well plate, Huh-7.5 cells were incubated with different concentrations of mAb16-71. After one hour the antibody was washed away and the Huh-7.5 cells were exposed to H77/JFH1 HCVcc, and infection was allowed to proceed for 48 hours. As shown in Figure 1a, a clear dose-dependent reduction of the amount of HCV infected cells was observed.

To evaluate the protective efficacy of mAb16-71 in a clinically more relevant *in vitro* model, we assessed antibody blockade in primary adult hepatocytes which, unlike hepatoma cells, are polarized and in which HCV entry factors localize to similar cellular compartments as in hepatocytes *in vivo* (38). Micropatterned co-cultures were pretreated with mAb16-71, anti-CD81 or isotype control antibody and subsequently infected with Jc1 HCVcc expressing *Gaussia* luciferase. As shown in Figure 1b, we observed a 5-fold reduction of HCV infection in the mAb16-71-treated wells compared to the isotype control; and a 7-fold reduction of infection upon anti-CD81 treatment. Though antibody C167, which has the same amino acid sequence as mAb16-71, has previously been demonstrated to be inefficient at blocking HCV entry in adult MPCC cultures, the robustness of HCV infection of these

cultures has since been improved. Additionally, donor variability could also help explain this difference (38). We confirmed our data in cultures of primary human fetal hepatocytes, which are a more amenable *in vitro* culture system, given the more robust infection levels achieved compared to adult hepatocytes (39). Primary human fetal hepatocytes, transduced with the RFPnls-IPS HCV reporter system (43), were pre-incubated with different concentrations of mAb16-71. Parallel cultures were treated with an isotype-matched antibody (negative control) or JS81, an antibody that targets CD81 and prevents attachment and infection of HCV (positive control). As shown in Figure 1c, infection of primary fetal hepatocytes by J6/JFH-1 HCVcc was also reduced in a dose-dependent manner.

mAb16-71 interferes with direct cell-to-cell transmission *in vitro*

HCV can spread directly from an infected Huh-7.5 cell to uninfected neighboring cells with possibly all four HCV entry factors CD81, SR-BI, claudin and occludin being involved in this process (32–34). However, by using Huh-7.5 target cells in which CD81 was selectively knocked down (EGFP-IPS/CD81^{neg}), we have recently shown that HCV cell-to-cell spread can occur independently of CD81 (43). We therefore used this cell line to investigate whether mAb16-71 is capable of inhibiting this alternative transmission route. To this end, HCVcc-infected (Jc1) Huh-7.5 cells were co-cultured with uninfected EGFP-IPS/CD81^{neg} cells. EGFP-IPS/CD81^{neg} cells have been previously shown to be essentially non-permissive to cell-free HCV infection (43). Nevertheless, mixing uninfected EGFP-IPS/CD81^{neg} target cells with infected Huh7.5 cells resulted in an infection of 8–10% of the target cells. However, in the presence of increasing concentrations of mAb16-71, a dose-dependent reduction in EGFP-IPS/CD81^{neg} target cell infection was observed, while no significant changes in HCV transmission were observed in the presence of an isotype-matched control antibody (Figure 1d). This clearly proves that mAb16-71 not only prevents cell-free HCV infection, but also interferes with the direct cell-to-cell transmission route.

Prophylactic administration of mAb16-71 protects chimeric mice from infection by HCV of different genotypes

Given the encouraging results in cell culture, we investigated whether administration of mAb16-71 to ‘human liver-uPA-SCID’ mice (chimeric mice) could protect these animals from a subsequent challenge with serum-derived virus. These mice have a humanized liver (up to 90% chimerism) and are in addition to the chimpanzee the preferred animal model for reproducible infection with natural HCV isolates (40, 42, 45, 46). Two chimeric mice underwent a two-week therapy consisting of five intraperitoneal injections, each containing 400 µg of mAb16-71. One day after the first injection, both chimeric mice were challenged with a 100% mouse infectious dose (MID₁₀₀) of serum-derived genotype 1a HCV (mH77C). In contrast to non-treated control animals, which experienced a rapid increase of viral RNA in their plasma, HCV RNA remained undetectable (<375 IU/ml) in both treated mice the 12-week observation period (Fig. 2a). mAb16-71 was equally effective in protecting five chimeric mice after challenge with serum-derived genotype 4 (mED43; n=3) or genotype 6 (mHK-6a; n=2) HCV, indicating that SR-BI-based immunotherapy is successful most likely irrespective of HCV genotype (Fig. 2b and c).

The two-week treatment protocol was very well tolerated by the chimeric mice, which showed no signs of overt toxicity. No significant changes in human albumin, transaminases (AST and ALT), triglyceride, cholesterol and HDL levels were measured in mice that received a two-week mAb16-71 therapy when compared to untreated control mice (Table 1).

Post-exposure mAb16-71 therapy blocks viral dissemination *in vivo*

To substantiate the role of SR-BI in cell-to-cell spread *in vivo*, we performed a post-exposure treatment experiment in chimeric mice. Fifteen chimeric mice were injected with a

MID₁₀₀ dose of mH77C HCV. Three days later, plasma HCV RNA levels were determined and HCV RNA could be detected in all but two animals that were included in the untreated group (n=7). Four of the remaining mice received five injections of mAb16-71 at day 3, 5, 7, 9 and 12 and the last four animals were treated with anti-CD81 antibody (clone JS81) using the same dosing protocol. In the untreated group the viral load rapidly increased during the first two weeks after virus inoculation, reaching values ranging between 10⁴ and 10⁷ IU/ml (Fig. 3a). Treatment with anti-CD81 mAb caused a minor, statistically non-significant, delay in the rise of viral load, possibly due to inhibition of infection by cell-free virus, but all animals experienced an increase in viral load, confirming our previous data that HCV can spread in a CD81-independent manner (31, 33). In contrast, in 3 out of 4 mice treated with mAb16-71, HCV RNA levels did not increase but remained positive at unquantifiable levels (<375 IU/ml), while in the 4th mouse HCV RNA was undetectable. In this mouse the viral load started to rise nine days after cessation of anti-SR-BI therapy and reached a level of almost 10⁶ IU/ml four weeks after infection (Fig. 3a). In the two other mAb16-71-treated mice the viraemia started to rise 16 to 23 days after cessation of therapy, while in the 4th mAb16-71-treated mouse HCV RNA remained detectable at unquantifiable levels throughout the 8-week observation period. Statistical analysis using the two-tailed non-parametric Mann-Whitney test showed that the median HCV RNA level of mAb16-71-treated animals differed significantly from that in the control group (P=0.023, P=0.0061 and P=0.016 at day 7, 14 and 21 respectively). No differences were observed between the HCV RNA levels of CD81-treated mice and control mice (P>0.99, P=0.164 and P=0.41 at day 7, 14 and 21 respectively). At the start of therapy (day 3) no statistically significant differences were observed between the different groups (control vs. mAb16-71: P=0.25; control vs. anti-CD81: P=0.45).

In the four mAb16-71-treated mice HCV RNA became detectable at different time points after cessation of therapy. Therefore we quantified the plasma mAb16-71 levels two days after the last antibody injection and observed a correlation between these mAb16-71 plasma levels and the duration of protection (Fig. 3b). High levels of circulating antibody indirectly indicate complete saturation of the SR-BI molecules present on the human hepatocytes in the chimeric mouse liver. In addition, sequence analysis of virus recovered from the mAb16-71-treated mice that became HCV positive at week 3 and week 5 showed that the deduced amino acid sequence of the envelope region was identical to the sequence of the viral inoculum and that of the viruses found in the control animals (data not shown). The absence of adaptive mutations and the correlation between plasma mAb16-71 levels and the duration of protection argue against virus escape.

A two-week mAb16-71 therapy of chronically infected chimeric mice had no effect on viral load (data not shown).

Discussion

Prevention of re-infection of the liver allograft in chronic HCV patients that underwent liver transplantation for end-stage liver disease (cirrhosis and/or hepatocellular carcinoma) will be one of the main therapeutic challenges of the next decade. New antiviral therapies consisting of pegylated interferon, ribavirin and protease inhibitors seem to be very effective in eradicating HCV infection in chronically infected patients without severe liver disease (6–8). However, these new antiviral cocktails elicit considerable side effects and the currently approved protease inhibitors are both inhibitors of cytochrome P450 3A, which is responsible for the metabolism of cyclosporine and tacrolimus (9, 10, 12). This will certainly severely complicate the use of telaprevir and boceprevir in a liver transplant setting. Because of the extreme variability of the viral envelope proteins and probably also because of the association of the viral particles with lipoproteins (47), anti-HCV antibodies

with neutralizing capacity hardly induce sterilizing immunity (13–17). Therefore, the genetically highly conserved cellular receptors utilized by the virus to infect the host cell may seem better alternatives to prevent infection of the allograft. Recently, Mensa et al. showed a correlation between the viral load decay during the first 24 hours after graft reperfusion and the SR-BI expression levels in the donor liver, suggesting that SR-BI plays a major role in the initial uptake of the virus and making it an attractive therapeutic target (48).

We have developed a human monoclonal antibody that efficiently prevents HCV infection of both Huh-7.5 hepatoma cells and primary hepatocytes. Moreover, this antibody is capable of interfering with direct cell-to-cell transmission of HCV *in vitro*. Importantly, using chimeric mice, we have clearly shown that anti-SR-BI therapy not only prevents infection by HCV of multiple genotypes, but it also inhibits the spread of the virus in an already established infection *in vivo*.

Antibody targeting of SR-BI turns out to be superior to anti-CD81 therapy for several reasons. The expression pattern of SR-BI is more restricted than the ubiquitously expressed CD81 (49), which may allow for a reduction of the treatment dose and potential side effects. In fact, a two-week treatment of chimeric mice with mAb16-71 induced no significant changes in hepatic serum markers as compared to untreated mice. Although our limited data suggest that mAb16-71 therapy might be safe in humans, more extensive preclinical toxicity studies must be performed in different animal species, as well as safety and pharmacokinetic studies in healthy volunteers and, ultimately, in liver transplant patients. It needs to be emphasized that mice represent a very stringent model for safety testing of anti-SR-BI mAb therapy since these animals do not express cholesteryl ester transfer protein (CETP) which facilitates cholesterol transport and triglyceride exchange in humans, thus potentially providing an alternative route of lipid metabolism in case of reduced SR-BI function upon mAb16-71 treatment (50).

In addition, mAb16-71 remains effective in blocking HCV dissemination, even if administered several days after viral inoculation. This suggests that SR-BI may be a molecule involved in direct cell-to-cell transmission of HCV *in vivo* and represents an important advantage over anti-CD81 blockade which did not prevent virus spread even when administered therapeutically soon after viral challenge (31). In fact, our antibody seems more effective *in vivo* than what could be anticipated from cell culture experiments. This implies still unknown discrepancies between the currently used cell culture systems and the *in vivo* reality, thereby further emphasizing the value of experiments in animal models.

Viruses with mutations or deletions in their envelope proteins have been described to become independent for SR-BI (51–53). However, it remains to be determined whether these mutated viruses are also not reliant on SR-BI *in vivo*. We could not identify any adaptive mutations in the envelope region of the virus that was recovered from two mAb16-71-treated mice that became HCV positive 9 days and 29 days after cessation of the two-week antibody treatment. Furthermore, it is doubtful that such variants would arise and expand in an infected patient, since they are sensitive to neutralizing antibodies that are ubiquitously present in the plasma of all chronically infected patients (13, 51–54). A viral mutant losing its SR-BI dependence would most likely be immediately neutralized by the host's pre-existing adaptive immune response.

Besides SR-BI, claudin-1 and occludin may be very attractive targets for antiviral therapy. These tight junction proteins are essential for viral entry and direct cell-to-cell transmission (29, 30, 34, 55). In a recent publication, Lupberger et al. identified the epidermal growth factor (EGF)-receptor as another host factor involved in HCV entry and dissemination (56).

However, a 30-day treatment of chimeric mice with Erlotinib, a small molecule that specifically inhibits EGF-receptor activity, did not prevent but only delayed the kinetics of infection.

In conclusion, we show here that the human monoclonal antibody mAb16-71 can efficiently block *in vitro* and *in vivo* infection by multiple HCV genotypes. In addition we demonstrate that blockade of SR-BI after infection can prevent rapid virus spread through the liver parenchyma, presumably by interfering with SR-BI-dependent cell-free as well as direct cell-to-cell HCV transmission. Therefore, targeting SR-BI may represent a superior strategy for anti-HCV immunotherapy to prevent the emergence of escape mutants and virus rebound during or following anti-viral therapy, and to prevent allograft infection in chronically infected HCV patients undergoing orthotopic liver transplantation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

IgG	immunoglobulin G
mAb	monoclonal antibody
SR-BI	scavenger receptor class B type I
HCVcc	cell culture produced HCV
efHeps	enriched fetal hepatocytes
TCID₅₀	50% tissue culture infectious dose
uPA	urokinase-type plasminogen activator
SCID	severe combined immune deficiency
MID₁₀₀	100% mouse infectious dose
AST	aspartate aminotransferase
ALT	alanine aminotransferase
HDL	high-density lipoprotein
CETP	cholesteryl ester transfer protein

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Figure 1. *In vitro* evaluation of neutralizing capacity of mAb16-71

(a) One day before infection, Huh-7.5 cells were seeded in 96-well plates at a density of 10^4 cells/well. The following day, the cultures were treated with increasing concentrations of the anti-SR-BI antibody mAb16-71. One hour later, the antibody solution was washed away and the cultures were infected with H77/JFH1 virus. Forty-eight hours post-infection, HCV-infected cells were visualized using an anti-NS5A antibody (9E10). For each antibody concentration, the number of infected cells in 34 microscopic fields (10X objective) is presented as the percentage of infected cells relative to the number of HCV-positive cells in non-treated cultures. The experiments were performed in triplicate and the error bars represent the standard error of the mean (SEM). **(b)** Primary adult human hepatocytes were maintained in micropatterned co-cultures (38) and pre-treated with isotype control antibody (20 μg/ml), mAb16-71 (2 μg/ml) or anti-CD81 (JS81, 2 μg/ml) and then infected with Jc1 GLuc virus. Infection was assessed by calculating the net luciferase activity produced between 24 hours and 48 hours after virus inoculation. All conditions were tested in duplicate and error bars represent the standard deviation. **(c)** Primary human fetal hepatocytes (39), plated in 24-well plates (4×10^5 cells/well), were transduced with the RFPnls-IPS HCV reporter system and pre-incubated with mAb16-71, JS81 or isotype-matched control antibody. HCVcc (3×10^6 TCID₅₀/well; J6/JFH1 clone 2) were added to the cells for 6 hours and unbound virus was washed away. Twenty-four hours later infected cells were counted using fluorescence imaging. All conditions were tested in duplicate and pictures were acquired in 5 random fields per well. Data is expressed as the mean % infected cells. Error bars represent the standard deviation. **(d)** EGFP-IPS/CD81^{neg} cells were co-cultured with Jc1-infected Huh-7.5 cells in the presence of increasing concentrations of anti-SR-BI antibody (0.02, 0.2 and 2 μg/ml) or isotype-matched irrelevant control antibody (2 μg/ml). After 72 hours, HCV-infected EGFP^{pos}-target cells were quantified by flow cytometry using AlexaFluor-647-conjugated 9E10 antibodies.

Figure 2. *In vivo* prevention of HCV infection

One day before infection, uPA-SCID mice harboring human hepatocytes in their liver (chimeric mice) were injected with 400 μ g of mAb16-71. Additional injections were given 1, 5, 8 and 12 days after inoculation of the virus. Chimeric mice were infected with serum-derived (a) mH77C (genotype 1a; n=2), (b) mED43 (genotype 4a; n=3) or (c) mHK6a (genotype 6a; n=2). Each red dot represents one individual treated chimeric mouse. HCV RNA present in mouse plasma was quantified via real-time RT-PCR (Roche TaqMan HCV test). LOD: limit of detection.

Figure 3. mAb16-71 treatment prevents the spread of an early HCV infection *in vivo* by preventing direct cell-to-cell transmission

(a) Chimeric mice were infected with serum-derived mH77C (genotype 1a). Three days later, all infected animals (n=15) were divided in three groups: one group received a 2-week mAb16-71 treatment (red, 4 animals), the second group was treated with anti-CD81 antibody (blue, 4 animals), while the third group served as an untreated control (black, 7 animals). HCV RNA present in mouse plasma was quantified via real-time RT-PCR (Roche TaqMan HCV test). LOQ: limit of quantification. (+) represents a non-quantifiable positive signal. **(b)** Two days after the last antibody injection, circulating mAb16-71 levels were quantified in the plasma of mAb16-71-treated mice. A clear correlation between circulating mAb16-71 plasma levels and the duration of protection was observed.

Table 1

Plasma analysis of control and mAb16-71 treated chimeric mice.

Treatment	Mouse ID	Hu Alb ¹ (mg/mL)		Triglycerides (mg/dL)		Cholesterol (mg/dL)		HDL (mg/dL)		AST (U/L)		ALT (U/L)	
		before	after	before	after	before	after	before	after	before	after	before	after
mAb16-71	B259L	9.3	13.3	190	123	88	144	22	85	162	183	45	44
	B186R	3.1	1.9	137	90	68	63	28	15	na ²	na	na	na
	B32	5.6	5.9	na	na	na	na	na	na	na	na	na	na
	K816LL	6.2	5.2	na	na	na	na	na	na	290	221	59	59
	K829RL	3.4	4.8	na	na	na	na	na	na	na	na	na	na
	B57	2.5	5.6	na	na	na	na	na	na	414	270	105	86
control	K983R	4.6	7.7	139	75	99	97	76	74	na	na	na	na
	K816L	5.1	4.5	na	na	na	na	na	na	351	333	98	137
	K816RL	3.6	na	na	na	na	na	na	na	489	461	89	131

¹ Hu Alb; human albumin

² na; data not available