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Humanized monoclonal antibody 2C9-cIgG has enhanced efficacy for yellow fever prophylaxis and therapy in an immunocompetent animal model

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ABSTRACT

Yellow fever virus (YFV) causes significant human disease and mortality in tropical regions of South and Central America and Africa, despite the availability of an effective vaccine. No specific therapy for YF is available. We previously showed that the humanized monoclonal antibody (MAb) 2C9-cIgG provided prophylactic and therapeutic protection from mortality in interferon receptor-deficient strain AG129 mice challenged with YF 17D-204 vaccine. In this study we tested the prophylactic and therapeutic efficacy of this MAb against virulent YFV infection in an immunocompetent hamster model. Intraperitoneal (ip) administration of a single dose of MAb 2C9-cIgG 24 h prior to YFV challenge resulted in significantly improved survival rates in animals treated with 380 or 38 μg of MAb compared to untreated animals. Treatment with the higher dose also resulted in significantly improved weight gain and reductions in serum alanine aminotransferase (ALT) and virus titers in serum and liver. Prophylactic treatment with 2C9-cIgG 24 h prior to virus challenge prevented the development of a virus-neutralizing antibody (vnAb) response in hamsters. Administration of a single ip dose of 380 μg of 2C9-cIgG as late as 72 h post-YFV challenge also resulted in significant improvement in survival rates. Hamsters treated at 4–72 h post-virus challenge developed a robust vnAb response. Enhanced survival and improvement of various disease parameters in the hamster model when MAb 2C9-cIgG is administered up to 3 days after virus challenge demonstrate the clinical potential of specific antibody therapy for YF.

1. Introduction

Infection with yellow fever virus (YFV) results in significant human morbidity and mortality in tropical regions of South and Central America and Africa. Like many other members of the *Flavivirus* genus, YFV is transmitted by mosquitoes. Although an effective vaccine is available for prevention of YF, it is estimated that 200,000 cases resulting in 30,000 deaths occur each year. Moreover, rare, severe vaccine-associated adverse events have been reported (Barrett and Teuwen, 2009), particularly in immunocompromised individuals. There is no approved treatment for cases of YF despite efforts to identify specific therapies (Julander, 2013).

Immune therapy, including passive administration of antibodies (Abs), is an effective method for treatment or prevention of infectious diseases (Keller and Stiehm, 2000). Limitations of this treatment are waning efficacy as the disease progresses, as well as difficulty in procuring sufficient quantities of human Ab for use in treatment. Humanization of mouse Ab combines the hypervariable regions of a murine monoclonal Ab (mMAb) gene specific to a given antigen with human Ab gene constant regions, and permits ease of production and use of clinically safe MAbs for the treatment of various human diseases.

The mMAb 2C9, which reacts with amino acids 71, 72, and 125 in domain II of the YFV envelope protein, has been shown previously to prophylactically protect 6-week-old mice from disease following intracerebral inoculation with YFV (Brandriss et al., 1986; Lobigs et al., 1987). We used MAb 2C9 to develop a human-mouse chimeric MAb, 2C9-cIgG, for evaluation of prophylactic and therapeutic efficacy for YFV infections.

Various animal models have been useful in the evaluation of investigational antiviral therapies for YF, including Ab treatment.

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Rhesus monkeys develop YF disease that is very similar to that observed in humans, although the course of disease tends to be more rapid, with death occurring within one week after infection (Monath et al., 1981). Rhesus monkeys were demonstrated to be useful models for immune therapy; they were protected from lethal YFV challenge by Ab administration up to 3 days post-infection (dpi), although immune serum treatment had no therapeutic effect if initiated after the onset of disease (Monath, 2008).

Mice typically develop encephalitis when infected with YFV, making them less well-suited as models of human disease. We recently developed a murine model of disease in mouse strain AG129, which is deficient in α/β- and γ-interferon receptors, peripherally challenged with YF 17D-204 vaccine (Thibodeaux et al., 2012a) and used this model system for evaluation of 2C9-cIgG (Thibodeaux et al., 2012b). Although AG129 mice lack a functional interferon response, they provide a useful model for initial proof of concept studies. We showed that both murine 2C9 and 2C9-cIgG protected AG129 mice from peripheral challenge with YFV 17D-204 when administered prophylactically 24 h prior to infection at antibody concentrations $\geqslant$1.27 μg/mouse and exhibited therapeutic activity when administered at 127 μg/mouse up to 24 h post-infection (hpi).

Infection of hamsters with the hamster-adapted Jimenez strain of YFV results in viscerotropic disease that is similar in many ways to human disease (Julander et al., 2007; Sbrana et al., 2006; Tesh et al., 2001; Xiao et al., 2001). Virus titer in serum peaks 4 days after virus challenge with mortality observed as early as 5 or 6 dpi. Relevant disease parameters, including increases in serum alanine aminotransferase (ALT), weight loss, and morbidity can be measured for evaluation of experimental antiviral therapeutic efficacy. This model has been used to evaluate a number of antiviral treatments, including passively administered neutralizing Abs (Julander et al., 2011). This hamster model was used in the present study to determine the efficacy of 2C9-cIgG in an immunocompetent and relevant model of YF.

2. Materials and methods

2.1. Animals

This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The study was conducted in accordance with protocol number 1231 approved by the Institutional Animal Care and Use Committee of Utah State University. Experiments were conducted in the AAALAC-accredited BSL-3 animal suite at the Utah State University Laboratory Animal Research Center (LARC). All LARC personnel continually receive special training on blood-borne pathogen handling by the university's Environmental Health and Safety Office. Female Syrian golden hamsters with an average weight of 99 g were used after a quarantine period greater than 48 h. Animals were randomly assigned to cages and individually marked with ear tags. Animals were observed at least twice daily for signs of morbidity and euthanized when severe morbidity occurred. Every effort was made to minimize suffering.

2.2. Antibody and virus

A stock of purified 2C9-cIgG was commercially prepared (QED Bioscience, San Diego, CA). The MAb was diluted in phosphate-buffered saline (PBS). A hamster dose was determined using results from the AG129 mouse studies (Thibodeaux et al., 2012b), based on surface area conversion. The West Nile virus (WNV)-specific, humanized MAb MGAWN1 was obtained from Macrogenics Inc. (Rockville, MD). Reagents were prepared in sterile saline immediately prior to initial administration and stored at 4 °C.

The hamster-adapted (p. 10) Jimenez strain of YFV was a generous gift from Robert B. Tesh (University of Texas Medical Branch, Galveston, TX). To prepare viral stocks, five adult female hamsters were infected by intraperitoneal (ip) inoculation of virus as described for the original hamster adaptation (Tesh et al., 2001). The livers of the infected hamsters were removed 3 dpi and homogenized in a 2× volume of sterile PBS. This liver homogenate supernatant had a titer of $10^{6.0}$ 50% cell culture infectious doses ($CCID_{50}$)/ml.

2.3. Animal challenge experiments

Although dose and timing of 2C9-cIgG administration differed between studies, all antiviral experiments had the same basic design. Hamsters were randomly assigned to groups of 5–21 animals. A single ip administration of 2C9-cIgG was used in all antiviral studies. The WNV-specific, humanized MAb hE16 (MGAWN1) (Oliphant et al., 2005) was included as a nonspecific MAb control in all studies at concentrations matching the highest dose of 2C9-cIgG. Ribavirin (provided by ICN Pharmaceuticals, Inc., Costa Mesa, CA), administered ip twice daily for 6 days beginning 4 h before infection at a dose of 50 mg/kg/d, was included in prophylaxis studies as a positive treatment control. Hamsters were challenged ip with 0.1 ml of virus administered bilaterally (0.2 ml total) at a virus concentration of $10^{2.0}$ $CCID_{50}$/ml (20 $CCID_{50}$/animal). Serum was collected 4 dpi to measure virus titer and from all surviving hamsters 6 dpi for quantitation of ALT. For quantitation of virus-neutralizing Ab (vnAb), serum was collected 4 h prior to virus challenge and at the termination of the study on 21 dpi. Hamsters were observed at least twice daily for morbidity, and weights were determined 0, 3, 5 and 6 dpi. Initial signs of morbidity occurred 4 dpi and moribund animals that were immobile were humanely euthanized. A group of mock-infected animals treated with 2C9-cIgG were included in the initial study as a toxicity control and uninfected, untreated normal controls were included in each experiment.

2.4. Infectious virus assays

Hamster liver homogenate supernatants and serum samples were serially diluted from 10^{-1} to 10^{-8} and 0.1 ml of each dilution was added to each of four wells of a 96-well plate containing confluent Vero 76 cells. Ten days later cytopathic effects (CPE) in each well were used to identify the end-point of infection for calculation of the $CCID_{50}$/ml. Results were expressed as $CCID_{50}$ per ml serum or g tissue for comparison.

2.5. Virus-neutralizing antibody assays

For quantitation of vnAb, a standard 50% plaque-reduction neutralization test ($PRNT_{50}$) was performed on Vero cells using previously published protocols (Julander et al., 2011).

2.6. Serum alanine aminotransferase assays

Serum was collected *via* bleeding from the ocular sinus 6 dpi. Alanine aminotransferase (ALT) SGPT reagent (Teco Diagnostics, Anaheim, CA) was used in the enzymatic assay, and the protocol was altered for use in 96-well plates as described previously (Julander et al., 2007). The ALT concentrations were determined per manufacturer's instructions.

2.7. Statistical analysis

Survival data were analyzed using the Wilcoxon log-rank survival analysis and all other statistical analyses were done with one-way ANOVA using a Bonferroni group comparison (Prism 5, GraphPad Software, Inc.).

3. Results

3.1. Prophylaxis of YFV infection with 2C9-cIgG

To verify efficacy of 2C9-cIgG in hamsters and to determine the effective dose range, a single ip injection of 380, 38, or 3.8 μg MAb was administered 24 h prior to YFV challenge. Treatment with 2C9-cIgG at the highest dose of 380 μg was well tolerated in mock-infected animals with no morbidity, as would have been indicated by severe weight loss, increase in ALT, or ruffled fur (data not shown). After virus challenge, a significant improvement in survival rates was observed at the 380 μg and 38 μg/hamster doses ($P < 0.01$ and $P < 0.05$, respectively) (Fig. 1). The lowest Ab dose of 3.8 μg protected 40% of the animals; however, this level of protection was not significantly different from the PBS control and was similar to survival of hamsters treated with WNV-specific MGAWN1 control MAb. Ribavirin, which was included as a positive treatment control, protected 60% of animals, which was significantly ($P < 0.01$) improved as compared with PBS treatment alone and yielded a survival rate similar to treatment with 38 μg of 2C9-cIgG (Fig. 1).

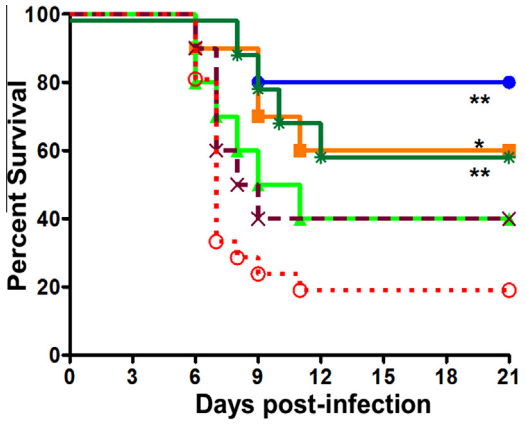

Fig. 1. Effect of prophylaxis with 2C9-cIgG on hamster survival from YFV challenge. MAb 2C9-cIgG was administered at 3 different doses 24 h before YFV challenge. Symbols: 380 μg (●), 38 μg (■), 3.8 μg (▲) of 2C9-cIgG/hamster; Ribavirin (*), MGAWN1 (×), PBS (○). Significance of increased survival rates was determined by comparison with PBS control (**$P < 0.01$, *$P < 0.05$). Sample sizes: $N = 10$ (380 μg), 10 (38 μg), and 10 (3.8 μg); 10 (Ribavirin), 10 (MGAWN1), 21 (PBS).

An increase in weight was observed in hamsters for the first week after treatment with 380 or 38 μg of 2C9-cIgG. This change was significant between 3 and 6 dpi as compared with PBS treatment ($P < 0.001$ and $P < 0.05$ for 380 or 38 μg, respectively) (Fig. 2A). Treatment with 3.8 μg of 2C9-cIgG did not result in significant weight increases. Interestingly, a significant ($P < 0.05$) improvement in weight change between 3 and 6 dpi was observed in animals treated with MGAWN1 MAb as compared with PBS treatment, although this improvement was not observed in therapeutic efficacy studies. Ribavirin treatment also resulted in an overall weight gain with significant ($P < 0.01$) improvement in weight change between 3 and 6 dpi as compared with PBS treatment (Fig. 2A). PBS-treated animals had a transient increase in weight (data not shown); however, weight declined after 3 dpi as is typical for hamsters infected with YFV.

Serum ALT levels at 6 dpi demonstrated a similar trend as weight change, with significant reduction ($P < 0.01$ and $P < 0.05$ for 380 or 38 μg, respectively) observed in animals treated with the two highest doses of 2C9-cIgG as compared with PBS treatment. Serum ALT levels of hamsters treated with 380 or 38 μg

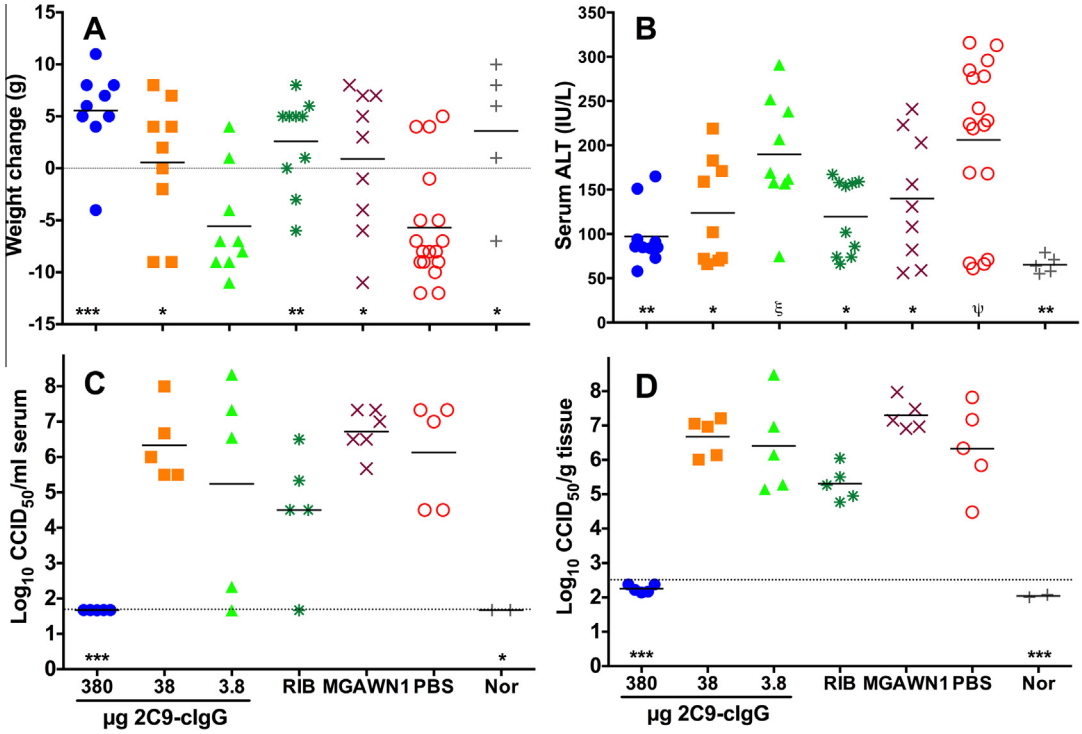

Fig. 2. Effect of prophylaxis with 2C9-cIgG on parameters of viral infection. (A) weight change from 3 to 6 dpi; (B) serum ALT at 4 dpi; (C) viral titer in serum at 4 dpi; (D) viral titer in liver at 4 dpi. Significance of differences as compared with PBS-treated controls is indicated: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$. Symbols and abbreviations: 380 μg (●), 38 μg (■), 3.8 μg (▲) of 2C9-cIgG/hamster; Ribavirin (*, RIB); MGAWN1 (X); PBS (O); normal uninfected, untreated controls (+, Nor). Significance of differences in serum ALT levels as compared with normal uninfected, untreated controls: ξ $P < 0.05$, ψ $P < 0.01$.

2C9-cIgG were not significantly different from those of untreated, uninfected animals (Fig. 2B) Ribavirin and MGAWN1 treatments also resulted in significant reduction of serum ALT levels as compared to PBS treatment.

Average virus titers in both serum and liver remained at background levels in the group treated with 380 μg of 2C9-cIgG (Fig. 2C and D, respectively). Despite significant improvement in survival, weight change, and serum ALT parameters, virus titers in animals treated with 38 μg of 2C9-cIgG or with Ribavirin were not significantly different from those of PBS-treated animals (Fig. 2C and D).

3.2. The effect of 2C9-cIgG prophylaxis on de novo virus-neutralizing Ab production

A $PRNT_{50}$ was used to quantify vnAb in the serum of animals from the prophylaxis study at 20 h after 2C9-cIgG administration and at 21 dpi to determine the effect of 2C9-cIgG treatment on the development of a *de novo* immune response. Only hamsters receiving the 380 μg 2C9-cIgG dose had measurable serum vnAb at 20 h after administration, likely representing 2C9-cIgG activity (Fig. 3A). Serum vnAb titers were at or below the level of detection at 21 dpi in surviving animals treated with 380 μg of 2C9-cIgG (Fig. 3B). The statistical comparison of vnAb levels between this treatment group and control groups was weakened by the low survival rate of the PBS-treated animals, but significant ($P < 0.001$) differences between animals treated with 380 μg of 2C9-cIgG and those treated with lower MAb doses or with Ribavirin were observed. No statistical differences were observed between the vnAb titers of animals in the 380 μg 2C9-cIgG treatment group and those from uninfected, untreated (normal control) animals. The vnAb titers at 21 dpi from animals treated with 38 μg or 3.8 μg of 2C9-cIgG were generally at or near the upper limits of detection in the $PRNT_{50}$ (Fig. 3B). A comparison between levels of vnAb from animals in the 38 and 3.8 μg 2C9-cIgG treatment groups to those from untreated, uninfected controls showed significant ($P < 0.001$ and $P < 0.01$, respectively) differences. The vnAb titers of animals treated with 50 mg/kg/d of Ribavirin or with 380 μg of MGAWN1 MAb also were near the upper limits of detection at 21 dpi (Fig. 3B).

The vnAb titers in 2 of 3 surviving animals from the PBS-treated group in the prophylaxis study were at or below the lower level of detection, suggesting little antigen exposure. Indeed, all surviving animals treated with PBS post-virus infection had vnAb titers that were close to or at the upper limit of detection (data not shown).

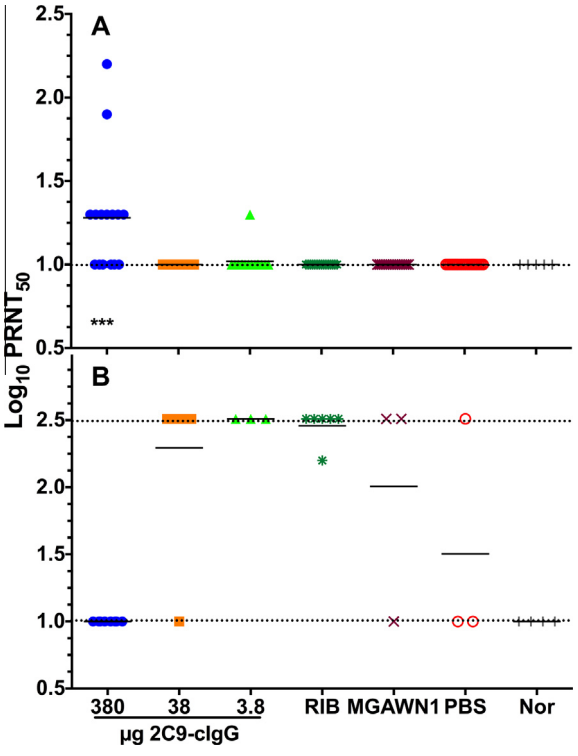

Fig. 3. Effect of 2C9-cIgG prophylaxis on hamster virus-neutralizing antibody response. Hamsters were treated 24 h before YFV challenge with doses shown of 2C9-cIgG, or with Ribavirin, MGAWN1, or PBS. Untreated, uninfected animals served as normal controls. Titers of vnAb in the serum were measured by $PRNT_{50}$ at (A) 20 h after treatment and 4 h prior to virus challenge and (B) 21 dpi. Symbols and abbreviations: 380 μg (●), 38 μg (■), 3.8 μg (▲) of 2C9-cIgG/hamster; Ribavirin (*, RIB); MGAWN1 (×), PBS (○), normal uninfected, untreated controls (+, Nor). Antibody titer differences that were significant as compared with PBS controls are indicated: $^{***}P < 0.001$. Dotted lines denote the upper and lower limits of detection.

3.3. Post-infection therapy with 2C9-cIgG

To determine the therapeutic efficacy of 2C9-cIgG, a single dose of 380 μg was administered to hamsters at 4, 24, or 48 h after virus challenge. Significant improvements in survival were observed after treatment at all 3 time-points (Fig. 4). Treatment at 48 hpi resulted in 100% survival, with earlier administration providing protection to 90% (9/10) of infected, treated animals, all of which were significant improvements over PBS treatment (Fig. 4). Treatment with MGAWN1 protected 40% of animals, which was not significantly different from PBS treatment.

Hamster weights increased linearly between 0 and 6 dpi in all animals treated with 2C9-cIgG regardless of treatment time (data not shown). While the increase was not as dramatic as that of untreated, uninfected (normal control) animals, the steady increase, as compared with a sharp decline observed in PBS-treated animals, was indicative of therapeutic efficacy. A significant ($P < 0.001$ for 4 and 24 h treatment, $P < 0.01$ for 48 h treatment) improvement in average weight change between 3 and 6 dpi was observed in all groups treated with 2C9-cIgG as compared with PBS or MGAWN1 treatment of animals infected with YFV (Fig. 5A).

Virus titers in serum of 2C9-cIgG-treated animals were significantly ($P < 0.01$) reduced at 4 dpi as compared with corresponding titers of PBS-treated controls (Fig. 5B). Serum titers in many of the samples were reduced to below the limit of detection. Treatment with MGAWN1 did not significantly reduce virus titers in serum

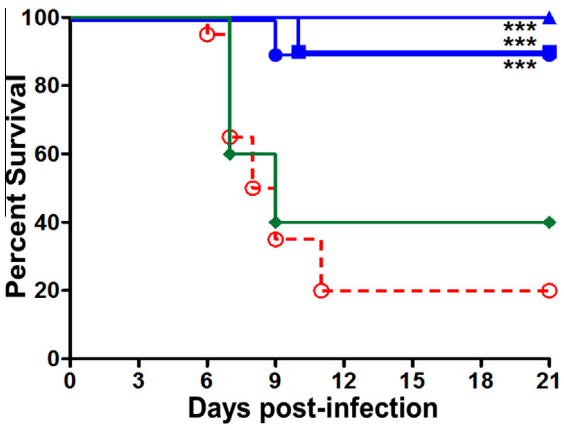

Fig. 4. Effect on hamster survival of treatment with a single dose of 380 μg of 2C9-cIgG at 4, 24, or 48 hpi. Symbols: administration of 2C9-cIgG at 4 hpi (●), 24 hpi (■), 48 hpi (▲), MGAWN1 at 4 hpi (♦), and PBS at 4 hpi (○). Sample sizes: $N = 10$ (4 h), 10 (24 h), and 10 (48 h), 10 (MGAWN1), and 20 (PBS). Significant differences in survival as compared with PBS are indicated: $^{***}P < 0.001$.

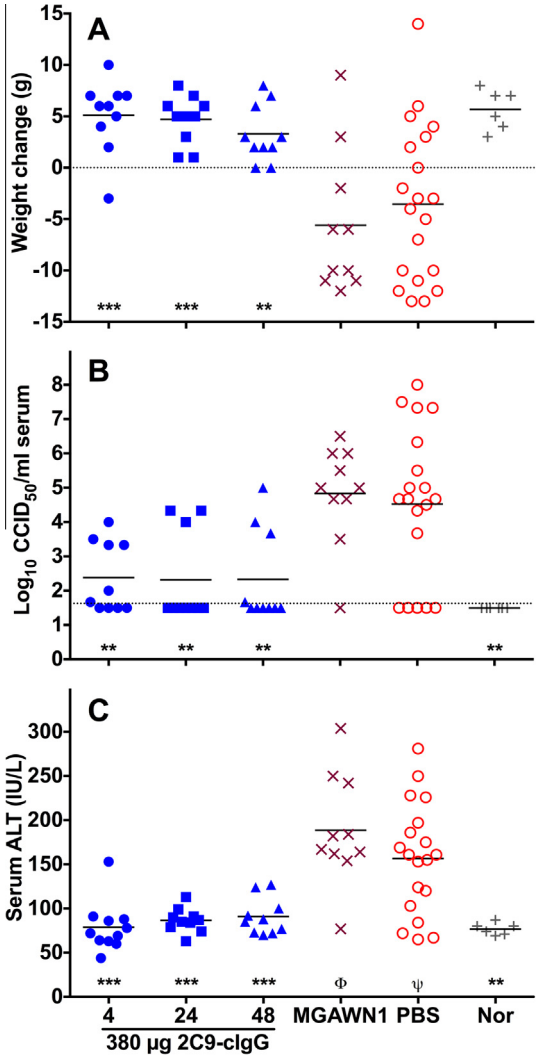

Fig. 5. Effect of treatment with a single dose of 380 µg of 2C9-cIgG at various times pi on parameters of viral infection. (A) weight change from 3 to 6 dpi; (B) viral titer in serum at 4 dpi; (C) serum ALT at 6 dpi. Symbols and abbreviations: 380 µg (●), 38 µg (■), 3.8 µg (▲) of 2C9-cIgG/hamster; MGAWN1 (×), PBS (○), normal uninfected, untreated controls (+, Nor). Significant differences as compared with PBS control are indicated: $^{***}P < 0.001$, $^{**}P < 0.01$. Significance of differences in serum ALT levels as compared with normal uninfected, untreated controls: ψ $P < 0.01$, φ $P < 0.001$.

and resulted in a slightly higher average titer than animals in the PBS treatment group (Fig. 5B).

Levels of serum ALT were also significantly ($P < 0.001$) reduced at 6 dpi in groups treated with 2C9-cIgG at 4, 24, and 48 hpi as compared with PBS treatment. Serum ALT levels in 2C9-cIgG-treated animals were similar to those of animals in the normal control group (Fig. 5C). Treatment with MGAWN1 did not reduce serum ALT levels, which were similar to those of animals treated with PBS.

Administration of 2C9-cIgG at 4, 24, or 48 hpi resulted in development of vnAb titers that were at or near the upper level of detection (data not shown). Similar values were also seen in surviving animals from the MGAWN1 or PBS treatment groups. No vnAb was detected in serum from uninfected control animals.

3.4. Temporal end-point for 2C9-cIgG therapeutic efficacy

A second post-challenge administration study was conducted to estimate the temporal endpoint of 2C9-cIgG therapeutic efficacy. A

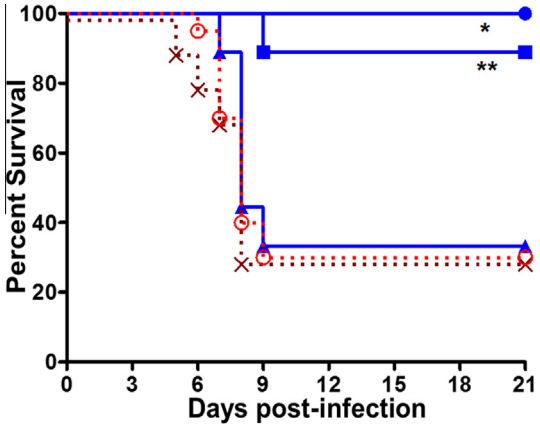

Fig. 6. Survival of hamsters treated with a single dose of 380 µg of 2C9-cIgG at 48, 72, or 96 hpi. Symbols: 48 hpi (●), 72 hpi (■), 96 hpi (▲), MGAWN1 at 48 hpi (×), PBS at 48 hpi (○). Significant differences in survival as compared with PBS-treated control are indicated: $^{**}P < 0.01$, $^{*}P < 0.05$. Sample sizes: $N = 5$ (48 hpi), 9 (72 hpi), and 9 (96 hpi), 10 (MGAWN1), and 20 (PBS).

single 380 µg dose of 2C9-cIgG was administered 48, 72, or 96 h after challenge of hamsters with YFV. Administration of 2C9-cIgG at 48 hpi was again shown to result in 100% survival (Fig. 6). Treatment with 2C9-cIgG at 72 hpi was also protective, resulting in a significant ($P < 0.01$) improvement in survival as compared with PBS or MGAWN1 treatment (Fig. 6). Treatment at 96 hpi was not effective and the proportion of animals surviving was similar to that of animals treated with MGAWN1 or with PBS (Fig. 6). Table 1 summarizes the prophylaxis and treatment regimens and rate of survival for each of the experiments shown in Figs. 1, 4 and 6.

Normal weight gain similar to that of uninfected, untreated controls was observed in animals treated with 2C9-cIgG at 48 hpi (Fig. 7A). Despite significant protection from mortality when 2C9-cIgG was administered at 72 hpi, weight loss was observed in these animals between 3 and 6 dpi, which was similar to that in animals treated 96 hpi and to PBS-treated infection controls.

Serum virus titers at 4 dpi were significantly ($P < 0.05$) reduced in animals treated with 2C9-cIgG at 48 hpi (Fig. 7B), which was consistent with increased survival rates. Animals treated at 72 or 96 hpi had viremia titers similar to those of animals treated with nonspecific control MAb MGAWN1 or PBS, indicating that later treatment does not significantly impact virus replication (Fig. 7B). Animals treated with 2C9-cIgG at 48 hpi had significantly ($P < 0.01$) reduced serum ALT levels as compared with PBS-treated animals (Fig. 7C), similar to those shown in Fig. 5C and not statistically different from levels in uninfected, untreated animals. However, no significant reduction in ALT of animals treated at 72 hpi was observed, despite an average serum ALT concentration lower than that of PBS-treated animals (Fig. 7C). Initiation of treatment at 96 hpi did not result in reduction of serum ALT.

4. Discussion

Passive Ab therapy is an important tool in the treatment of human infectious diseases (Casadevall et al., 2004). Since there are no approved human therapies for the treatment of acute flavivirus infections, including YFV, studies to determine the potential of Ab therapy for YF in clinically relevant animal models are needed. The hamster model of YF used in these studies was developed by Tesh et al. (2001) after they observed that YFV infection in these animals exhibited similarities to viscerotropic disease manifestations in natural human YFV infections.

Table 1
Summary of protocols and survival rates for prophylaxis and therapy experiments.

Experiment	YFV infection	Treatment administered	Time of treatment admin.	Number of animals (survivors/total)
Prophylaxis	Yes	380 μg 2C9-cIgG	24 h prechallenge	8/10
	Yes	38 μg 2C9-cIgG		6/10
	Yes	3.8 μg 2C9-cIgG		4/10
	Yes	MGAWN1		4/10
	Yes	PBS		4/21
	Yes	Ribavirin	4 h pre, 2x/d post	6/10
	No	None	–	5/5
Therapy	Yes	380 μg 2C9-cIgG	4 hpi	9/10
	Yes		24 hpi	9/10
	Yes		48 hpi	15/15 (2 expts)
	Yes		72 hpi	8/9
	Yes		96 hpi	3/9
	Yes	MGAWN1	4 hpi	4/10
	Yes		48 hpi	3/10
	Yes	PBS	4 hpi	4/20
	Yes		48 hpi	6/20
	No	None	–	11/11 (2 expts)

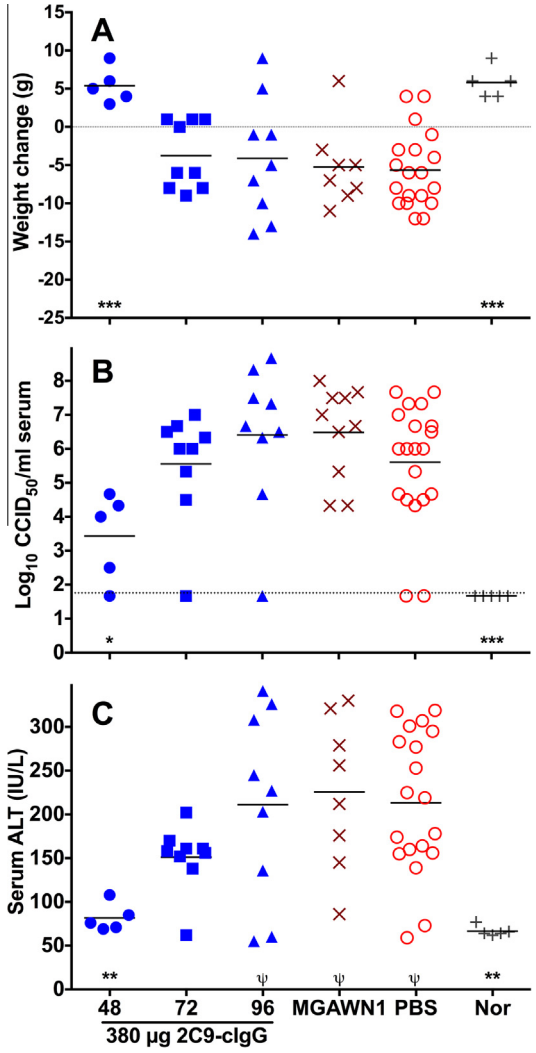

Fig. 7. Effect of treatment with a single dose of 380 μg of 2C9-cIgG at 48, 72, or 96 hpi on parameters of viral infection. (A) weight change from 3 to 6 dpi; (B) viral titer in serum 4 dpi; (C) serum ALT at 6 dpi. Symbols and abbreviations: 380 μg (●), 38 μg (■), 3.8 μg (▲) of 2C9-cIgG/hamster; MGAWN1 (×), PBS (○), normal uninfected, untreated controls (+, Nor). Significant differences as compared with PBS-treated control are indicated: $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$. Significance of differences in serum ALT levels as compared with normal uninfected, untreated controls: ψ $P < 0.01$.

We previously showed that mMAb 2C9 and 2C9-cIgG therapy in YFV-17D-204-infected AG129 mice, which lack interferon-α/β and -γ receptors, led to increases in survival rates only when administered at or before 24 hpi (Thibodeaux et al., 2012b). This immunocompromised mouse strain is highly susceptible to challenge with vaccine strain YFV, which replicates efficiently and causes both viscerotropic disease and CNS infection (Thibodeaux et al., 2012a). Despite dissimilarity to human disease, the YFV-17D-AG129 mouse model has the advantage of lower biosafety-level requirements than virulent YFV, underscoring its utility in initial antiviral studies. In addition, use of the AG129 mouse model for antiviral studies may give valuable insights into use of immunotherapy for immune-compromised humans. Developing treatment for post-vaccinal adverse events requires an animal model in which disease results from peripheral inoculation of YFV vaccine, such as AG129 mice.

In this study, our major focus was to determine if prophylactic and/or therapeutic administration of 2C9-cIgG in an immunocompetent animal model would provide protection from mortality sufficient to allow the host to mount an effective immune response. We demonstrated that treatment of hamsters with 2C9-cIgG from 24 h before infection up to 72 hpi was effective in significantly reducing mortality rates. Treatment at 72 hpi coincides with the time just prior to overt signs of illness, which may be apparent as early as 4 dpi. Peak viremia and liver virus titers are observed at 4 dpi and mortality may occur as early as 5 dpi (Julander et al., 2007). These results are consistent with our previous study in which passive transfer of serum collected from hamsters immunized with an inactivated YFV vaccine resulted in significant protection against virus challenge in the hamster model (Julander et al., 2011). The prolonged time period in which therapeutic administration of 2C9-cIgG resulted in significant improvement in survival shown in the current study with virulent YFV infection of immunocompetent hamsters emphasizes the importance of the host immune response in augmenting antiviral therapy. The hamster model is better suited than the AG129 mouse model for therapeutic efficacy studies that represent treatment of YFV infection of immunocompetent humans.

Levels of vnAb were low or absent at 21 dpi in hamsters treated with high doses of 2C9-cIgG 24 h prior to virus challenge, suggesting that prophylactic MAb administration resulted in sterile immunity in the hamsters. This has also been observed in other YFV studies after effective prophylactic treatment (Julander et al., manuscript in preparation). Administration of 380 μg 2C9-cIgG up to 72 h, but not 96 h, after virus challenge provided significant

protection from mortality and permitted development of a vnAb response in surviving hamsters.

The hamster model manifests virologic and clinical parameters similar to YF in humans such as elevated serum ALT, which is a specific marker for liver necrosis, and failure to gain weight, a sign of morbidity due to appetite loss. We examined these parameters in this study to further characterize efficacy of immunotherapy. Administration of 380 or 38 μg of 2C9-cIgG 24 h before YFV challenge resulted in weight gains between 3 and 6 dpi that were significantly higher than those of PBS-treated animals. Similarly, hamsters treated with these higher doses of prophylactic 2C9-cIgG exhibited serum ALT levels that were not significantly different from those of normal, uninfected animals, suggesting protection from hepatic necrosis. Unexpectedly, despite mortality rates not significantly different from animals treated with PBS, mean weight gain and serum ALT levels in hamsters treated with the nonspecific MAb MGAWN1 at 24 h pre-challenge were significantly improved compared to PBS-treated animals, but values varied over wider ranges than those of the 38 μg 2C9-cIgG-treated animals. Variability in individual hamster responses to virulent YFV infection, including serum ALT levels and mortality rates, has been observed in previous studies (Tesh et al., 2001; Sbrana et al., 2006; Julander et al., 2007), so these observations might simply reflect this variability. Significant improvements in weight gain and serum ALT levels were not seen in MGAWN1-treated hamsters in subsequent therapeutic studies.

Corresponding to the apparent sterile immunity conferred by prophylactic administration of 380 μg 2C9-cIgG, hamsters that received this treatment had undetectable infectious virus in sera and livers at 4 dpi, although hamsters that received lower doses of prophylactic MAb developed viremia and liver titers not significantly different from PBS-treated controls, despite apparent protection from liver damage demonstrated by normal serum ALT levels.

Animals treated with 380 μg 2C9-cIgG at 48 hpi had significantly reduced viremia titers at 4 dpi and serum ALT levels at 6 dpi compared to PBS-treated animals; however, although administration of therapeutic MAb up to 72 hpi provided significant protection from mortality, these animals exhibited loss of weight between 3 and 6 dpi and infectious virus titers and ALT levels in sera were not significantly different from PBS-treated hamsters, suggesting active YFV replication and tissue damage.

Our findings in this and our previous study suggest there is a potential for clinical use of humanized MAb during an outbreak of YF, particularly before virus exposure to prevent infection, as well as in treatment of naturally YFV-exposed individuals early in infection or those who experience adverse reactions to YFV-17D vaccination.

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