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A small animal peripheral challenge model of yellow fever using interferon-receptor deficient mice and the 17D-204 vaccine strain

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Abstract

Yellow fever virus (YFV), a member of the genus *Flavivirus*, is a mosquito-borne pathogen that requires wild-type (wt), virulent strains be handled at biosafety level (BSL) 3, with HEPA-filtration of room air exhaust (BSL3+). YFV is found in tropical regions of Africa and South America and causes severe hepatic disease and death in humans. Despite the availability of effective vaccines (17D-204 or 17DD), YFV is still responsible for an estimated 200,000 cases of illness and 30,000 deaths annually. Besides vaccination, there are no other prophylactic or therapeutic strategies approved for use in human YF. Current small animal models of YF require either intra-cranial inoculation of YF vaccine to establish infection, or use of wt strains (*e.g.*, Asibi) in order to achieve pathology. We have developed and characterized a BSL2, adult mouse peripheral challenge model for YFV infection in mice lacking receptors for interferons α , β , and γ (strain AG129). Intraperitoneal challenge of AG129 mice with 17D-204 is a uniformly lethal in a dose-dependent manner, and 17D-204-infected AG129 mice exhibit high viral titers in both brain and liver suggesting this infection is both neurotropic and viscerotropic. Furthermore the use of a mouse model permitted the construction of a 59-biomarker Multi-Analyte Profile (MAP) using samples of brain, liver, and serum taken at multiple time points over the course of infection. This MAP serves as a baseline for evaluating novel therapeutics and their effect on disease progression. Changes (4-fold or greater) in serum and tissue levels of pro- and anti-inflammatory mediators as well as other factors associated with tissue damage were noted in AG129 mice infected with 17D-204 as compared to mock-infected control animals.

Keywords

yellow fever; AG129 mice; animal models

INTRODUCTION

Yellow fever virus (YFV), a member of the genus *Flavivirus*, family *Flaviviridae*, is found in tropical regions of Africa and South America and is transmitted to primates by

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mosquitoes. Despite the availability of effective vaccines, YF is still a significant public health concern - responsible for an estimated 200,000 cases and 30,000 deaths annually - and is considered a re-emerging disease. This re-emergence is due to reinfestation of countries with the *Aedes aegypti* vector, lapses in implementation of preventative vaccination programs in endemic regions such as sub-Saharan Africa, and the lack of vaccination compliance by at-risk populations [1, 2, 3, 4]. Given the increase in travel and commerce originating from once isolated YFV-endemic regions, there is a growing concern regarding the potential of YFV to cause urban epidemics if introduced into geographical regions containing the *Ae. aegypti* mosquito vector and large concentrations of susceptible people (e.g., the southern U.S.) [5, 6].

For those with access to vaccination, the use of the live-attenuated YF vaccine is contraindicated in certain individuals. It is advised that infants under 6 months of age not be given the YF vaccine due to a risk of viral encephalitis [7]. Also at risk are those individuals who suffer from hypersensitivity to eggs, since the YF vaccine is prepared in embryonated eggs. The YF vaccine is not recommended for those who are immunocompromised due to AIDS or HIV infection, or whose immune system has been altered by either diseases such as leukemia and lymphoma or through drugs and radiation [7]. Studies have shown that persons aged ≥ 65 years are particularly susceptible to systemic adverse events following immunization with YF 17D-204 [2, 8, 9].

There are no approved therapeutic drugs for YF, thus treatment is primarily supportive and rarely modifies disease outcome [10, 11]. Early *in vivo* studies of YFV infection and therapy in mouse models were hindered by the need for intra-cranial (i.c.) inoculation of virus to establish infection [12,13]. Although the i.c. route of virus challenge guarantees infection, it is not a realistic model of viral challenge because YFV is normally transmitted peripherally to humans via mosquito-bite. Furthermore, YFV is considered a hepatotropic virus and although encephalitis resulting from viral replication in the brain is occasionally observed in natural YF infections, the i.c. route of virus delivery eliminates the opportunity to study YFV-associated hepatic damage. More recent small animal models of YFV infection are able to simulate viscerotropic infection using golden Syrian hamsters or mice with impaired Type I interferon (IFN) α/β responses [14, 15]. However both models require the use of wild-type (wt) virulent strains of YFV, e.g., Asibi, that require ABSL3+ containment, thus limiting facilities in which studies using these models can be carried out. Our search for a suitable small animal peripheral challenge model for dengue virus (DENV), another member of the *Flavivirus* genus, revealed that peripheral challenge of AG129 mice (129/Sv/Ev, deficient in combined IFN α/β and γ receptors) with DENV resulted in morbidity and mortality [16, 17]. Previous studies have reported on the morbidity resulting from infection of AG129 mice with 17D-204; however, detailed analyses of viral growth kinetics were limited to the early stages of infection. One such study by Lee and Lobigs [18] demonstrated that subcutaneous (s.c) inoculation of AG129 mice with 3 log PFU of 17D-204 resulted in 88% mortality with an average survival time (AST) of 18.1 ± 2.4 days post-infection (p.i.). Infectious virus titers in spleens and other tissues were dose-dependent and were monitored up to day 7 p.i. A similar study by Meier et al. [15] found that AG129 mice infected s.c. with 4 log PFU of 17D-204 experienced a shorter average survival time (10.1 ± 1.4 days p.i.) than that observed by Lee and Lobigs.

Here we characterize in greater detail the infection of AG129 mice with 17D-204, extending the findings of previous researchers regarding effective doses and routes of challenge for infection as well as viral growth kinetics in multiple tissues throughout infection. Furthermore, we have generated a comprehensive biomarker profile of 59 separate analytes associated with inflammation and tissue destruction. This information provides a highly-

detailed model of YF disease that can be used for the study of novel therapeutics and serving as a baseline for studying immunopathology associated with flavivirus infection.

MATERIALS AND METHODS

Mice

The 129/Sv/Ev mice deficient for both IFN- α/β and γ receptors (strain AG129) obtained from B & K Universal (Hull, United Kingdom) and housed in the Division of Vector-Borne Diseases (DVBD) animal care facilities at CDC were used for all animal studies [17]. Mice were euthanized with isoflurane followed by cervical dislocation when signs of illness became obvious as indicated by reduced activity and increased huddling during normal activity hours, lack of appetite, and the development of neurologic signs such as hind leg paralysis or weakness. The use of animals for research purposes complied with all relevant federal guidelines and specific protocols were approved by the DVBD Institutional Animal Care and Use Committee.

Cell Lines and virus

The YFV-17D vaccine strain (17D-204) was obtained from the DVBD reference collection (Fort Collins, CO). A single pool containing 2.0×10^7 plaque-forming units per ml (PFU/ml; verified by assay on Vero cell monolayers) of 17D-204 grown in Vero cells with minimum essential medium (MEM; D-MEM supplemented with 10% FBS, 2 mM L-glutamine, 0.15% sodium bicarbonate, 100 U/ml penicillin G sodium, and 100 μ g/ml streptomycin sulfate) was used for all inoculations.

Animal studies

Mice used in all challenge experiments were between 6 and 7 weeks of age. All mice challenged with 17D-204 received a single 100 μ l intraperitoneal (i.p.) injection or single 10 μ l subcutaneous (s.c.) injection in the region of the footpad at varying doses (see below).

Effective challenge dose—Groups of AG129 mice ($n \geq 5$ /group) were challenged with varying doses of 17D-204 in order to determine the dose that resulted in morbidity in 100% of the challenged mice. Ten-fold dilution challenge doses of 17D-204 corresponding to inoculum titers of 2×10^1 to 2×10^6 PFU/mouse were prepared in MEM. A separate group of mice were inoculated with MEM alone as a negative control. Mice exhibiting signs of morbidity (as described above) were euthanized and blood was collected post-mortem via cardiac puncture; serum was separated using microtainer tubes (Becton-Dickinson, Franklin Lakes, NJ) and analyzed for murine anti-YFV IgM antibodies by MAC-ELISA as previously described [19] to confirm viral infection.

Growth curve—AG129 mice ($n = 25$) were challenged with 2×10^5 PFU/mouse 17D-204 in order to determine the viral burden in select tissues during the course of the infection. Between days 1 and 11 p.i. groups of mice ($n = 3$) were sacrificed every 2 days; beginning at day 12 p.i., which coincided with the first appearance of morbidity, mice were euthanized upon exhibiting signs of morbidity. Tissue samples were collected post-mortem; blood samples were placed directly into microtainer tubes for serum collection and liver, brain, kidney, and spleen samples were rinsed in PBS, manually chopped into small sections, and placed into pre-weighed MagNA Lyser Green Bead tubes (Roche Diagnostics GmbH, Mannheim, Germany) containing 500 μ l MEM (0% FBS). Tubes containing tissue samples were immediately weighed and subsequently homogenized for two 30 s cycles at 4,000 rpm using a Roche MagNA Lyser (Roche Diagnostics GmbH). Homogenized samples were centrifuged for 5 min at 10,000 rpm at room temperature (RT) and stored at -70°C along with serum samples for later RNA purification.

Rodent multi-analyte profile (MAP)—AG129 mice (n = 24) were challenged with 2×10^5 PFU/mouse 17D-204; additional AG129 mice (n = 6) were injected with MEM to serve as a mock-challenged control group. Beginning at day 1 p.i. and continuing every 2 days up to and including day 15 p.i. mice (n = 3) were sacrificed and blood, liver, and brain samples were collected from infected animals post-mortem; tissues were also collected from mock-infected animals at day 7 and day 15 p.i. (n = 3/time point). Serum and tissue samples were processed as described above with the exception that approximately half of each brain or liver sample was placed into a Green Bead tube containing 500 μ l MEM (0% FBS). The remaining half of each tissue sample was placed into a Green Bead tube containing 500 μ l of TE buffer (2 mM EDTA, 50 mM Tris-HCl, pH 7.4) and the following protease inhibitors: 1 mg/ml of Pefabloc SC (Roche) and 1 μ g/ml each of aprotinin, antipain, leupeptin, and pepstatin (Roche). Serum samples and supernatants of liver or brain homogenates from each group were pooled (n = 3 samples/pool) and frozen at -70°C . Pooled samples were subsequently shipped to Rules-Based Medicine, Inc. (RBM, Austin, TX) for analysis of 59 biomarkers on the RodentMAP® version 2.0 platform. The RodentMAP® v2.0 platform employs microspheres impregnated with fluorescent dyes and coated with reagents that bind with target substances in serum or tissue homogenates. The least detectable dose (LDD) is defined as 3 standard deviations above mean background measured for each analyte in each multiplex.

Histopathology—Three mice infected i.p. with 17D-204 were euthanized at days 6 and 12 p.i.. Samples of liver tissue removed from these mice were fixed in 10% formalin and tissues were sent to Colorado Histo-Prep (Fort Collins, CO) for paraffin-embedding, sectioning and hematoxylin and eosin (H&E) staining.

Viral quantitation

RNA extraction from tissues—For determination of viral genome content of mouse tissues, frozen samples of brain, liver, kidney or spleen were thawed and total RNA was purified from 50 μ l of clarified homogenate using an RNeasy 96 Universal Kit (Qiagen, Valencia, CA) following the manufacturer's protocol; RNA was purified concurrently from ten-fold serially-diluted 17D-204 viral seed using the RNeasy 96 Universal Kit for generation of a standard curve. The same serially-diluted 17D-204 viral seed was also titrated by plaque assay to correlate viral genomic equivalents with PFU/ml. Viral RNA was purified from serum samples using a QIAamp® Viral RNA Mini Kit (Qiagen) following the manufacturer's protocol.

Quantitative reverse-transcription polymerase chain reaction (qRT-PCR)—The forward primer (8280f), reverse primer (8354r) and fluorogenic probe (8308pr, 5' 6-carboxyfluorescein *FAM+ reporter dye and 3' black hole quencher 1 [BHQ1]) used for qRT-PCR were obtained from the CDC Biotechnology Core Facility (Atlanta, GA): 8280f CCACTCATGAAATGTACTACGTGTCTG, 8354r GGAGGCGGGATGTTTGTT, 8308pr AGCCCGCAGCAATGTCACATTTACTGT. Each reaction mixture contained 5 μ l of purified RNA; primers and probes were used at final concentrations of 1 μ M for 8280f and 8354r and 0.2 μ M for the 8308pr. Amplifications were performed in an iQ 5 Real-Time PCR Detection System (Bio-Rad, Hercules, CA) using a QuantiTect® Probe RT-PCR Kit (Qiagen) under the following conditions: 50°C for 30 minutes, 95°C for 12 minutes 30 seconds, followed by 45 cycles of 94°C for 15 sec, 55°C for 1 min with continuous fluorescence data collection. Each RNA sample was tested in duplicate and virus genome equivalents were determined by extrapolation from the standard curves generated within each experiment.

Viral plaque titration—The 17D-204 viral seed was serially diluted (ten-fold) in MEM and adsorbed for 1 h at 37°C onto Vero cell monolayers in six-well plates, and overlaid with 3 ml media containing 1% agarose. Plates were incubated at 37°C for 4 days, after which 3 ml of a second overlay, containing the ingredients of the first overlay with neutral red (Sigma) added to a final concentration of 0.0053%, was added. Plates were incubated at 37°C for an additional 24 h before plaque formation was recorded.

RESULTS

Determination of an effective challenge route and dose of 17D-204 in AG129 mice

We have previously determined that AG129 mice lacking an IFN response were susceptible to peripheral challenge with DENV [16]. Furthermore, studies by Meier et al. [15] and Lee and Lobigs [18] demonstrated that AG129 mice are uniformly susceptible to infection with 17D-204 vaccine inoculated into the footpad at doses ranging from 10^3 to 10^5 PFU. For these reasons we examined AG129 mice as a possible host for a peripheral challenge model of YFV infection. Challenge of AG129 mice via i.p. injection with 17D-204 at doses ranging between 10^4 – 10^6 PFU resulted in 100% morbidity. Onset of morbidity in infected mice occurred in a dose-dependent fashion, with mice receiving 10^6 PFU showing signs of morbidity as early as day 7 p.i., whereas the appearance of morbidity in mice receiving 10^5 or 10^4 PFU of 17D-204 occurred at days 9 and 16 p.i., respectively (Figure 1). The AST of mice challenged peripherally with 17D-204 was also dose-dependent with higher titered inocula correlating with shorter AST (Table 1). Not surprisingly, lower doses (10^1 to 10^3 PFU) resulted in incomplete morbidity of challenged animals (data not shown).

AG129 mice were challenged in a separate experiment via s.c. injection in the region of the footpad with 4 or 5 log PFU of 17D-204; s.c. challenge of mice with 5 log PFU resulted in 100% mortality with an AST of 16.8 days and those challenged with 4 log PFU exhibited an AST of 20.8 days; however, mortality in this group was only 70% (Table 1). Given these findings, a challenge dose of 10^5 PFU delivered i.p. was used in all subsequent studies for experimental convenience and maximum stringency.

Determination of viral load in 17D-204-infected AG129 mouse tissues

An analysis of viral replication in multiple tissues of AG129 mice challenged with 10^5 PFU of 17D-204 revealed that viremia peaked at day 5 p.i. with a mean titer of $3.91 \pm 0.47 \log_{10}$ genomic copies (GC)/ml and dropped to nearly undetectable levels ($< 1.0 \log_{10}$ GC/ml) thereafter (Figure 2a). Viral burden in the spleen peaked by day 3 PI ($7.13 \pm 0.22 \log_{10}$ GC/gram) and remained relatively high ($4 - 6 \log_{10}$ GC/gram) throughout the infection (Figure 2b). Viral burden in the brain rose steadily during the course of the infection, peaking between days 14 – 16 p.i. ($7-8 \log_{10}$ GC/gram) (Figure 2c) while viral burden in the liver and kidney peaked earlier (3 days p.i.; liver: $4.69 \pm 0.31 \log_{10}$ GC/gram, kidney: $4.96 \pm 0.40 \log_{10}$ GC/gram) and began to drop shortly thereafter (Figures 2d and 2e). At time of sacrifice, the 17D-204 titers in livers and kidneys of the majority of infected mice had decreased to non-detectable levels (Figures 2d and 2e). These findings indicate that peripheral infection of AG129 mice with 17D-204 results in a lethal neurotropic infection with a transient viscerotropic phenotype.

H&E-stained brain and liver sections taken at 6 and 12 days p.i. from AG129 mice infected with 17D-204 were analyzed independently by two separate veterinary pathologists using light microscopy. Similar to previous studies with 17D-204-infected AG129 mice [15], no apparent tissue damage in the liver was observed in this study (data not shown).

Construction of 59-analyte biomarker profile

Pooled samples of serum or tissue homogenate supernatants (brain and liver) were analyzed using the RodentMAP® v2.0 platform in order to construct a biomarker profile of the AG129/17D-204 model throughout the infection. Baseline measurements for each analyte in mock-infected animals (AG129 mice receiving sterile MEM) were calculated by averaging the results of two separate readings taken at 7 and 15 days p.i.. Overall, brain tissue homogenates from 17D-204 infected AG129 mice showed 4-fold or greater differences in concentration in the largest number of analytes (36 of 59; 29 analytes increasing [$\uparrow$] and 7 analytes decreasing [$\downarrow$]) compared to mock-infected animals. Liver homogenates had the second highest overall number of analytes with 4-fold differences (20 of 59; 17 $\uparrow$ and 3 $\downarrow$); serum samples had the lowest (13 of 59; 11 $\uparrow$ and 2 $\downarrow$). Table 2 contains a summary of those analytes from AG129 mice that exhibited a 4-fold or greater change in concentration as a result of 17D-204 infection compared to mock-infected animals; for the sake of brevity, only those analytes associated with inflammation or tissue damage are shown; a more detailed report of the concentration of each analyte in each sample throughout the course of the infection can be found in Supplemental Table 1. In brain homogenates, the majority of analytes undergoing 4-fold changes in concentration demonstrated increasing concentrations as the infection progressed, a pattern that mimicked viral burden in the brain (Figure 3a). A similar association between viral tissue titer and analyte concentration was observed in the liver, with most analytes reaching peak concentrations on the same days that viral liver titers were highest (days 3 or 5 p.i.) and then decreasing shortly thereafter. Unlike viral liver titers, however, a surprising number of liver analytes experienced a second peak on day 11 p.i., a point at which virus had been cleared from the liver (below 2 log₁₀ GC/gram)(Figure 3b). Analyte concentrations in the serum followed a similar pattern to those in the liver, including the second peak on day 11, although to a much smaller degree (Figure 3c).

DISCUSSION

The study of YF disease and the development of novel antiviral therapies are hindered by the lack of an appropriate small animal model of YF. For many years the YF murine model required that virus be delivered by i.c. inoculation or that very young mice be used prior to their acquisition of YFV-resistance as their immune response matured. We previously determined that the IFN response-deficient AG129 mouse (129Sv[ev] background) was particularly susceptible to flavivirus infection. This observation demonstrated for the first time the critical contributions of the IFN response to flavivirus immunity [16]. Since then, the AG129 mouse has been used to examine some aspects of YF disease due to its susceptibility to peripheral inoculation of both the wt virulent Asibi strain and the 17D-204 vaccine strain. Lee & Lobigs originally reported that AG129 mice were uniformly susceptible to infection with 17D-204, resulting in lethal neurotropic infection [18]. More recently, a study by Meier et al. demonstrated that infection of these mice with Asibi resulted in viscerotropic pathology similar to YF disease in humans [15]. Here we report in greater detail on the replication kinetics of 17D-204 in various tissues of AG129 mice. In addition, the concentrations of 59 separate analytes in brain, liver, and serum have been tracked throughout the course of the infection. When combined with the viral growth kinetics data in these tissues, this characterization provides a powerful tool for future studies evaluating the inflammatory response following vaccination as well as the effectiveness of novel therapeutics.

The AG129/17D-204 model described in this study allows for a broader morbidity window in which to administer antibodies or other therapeutics and observe any protective effects compared to the AG129-Asibi model where the AST of infected mice is 6.4 days [15]. Although appropriate for those studies, previous investigations of AG129 infected with

17D-204 focused on viral burden in tissues for only 3 – 6 days p.i. We more completely defined the viral loads in infected tissues throughout the entire infection in this study.

Additionally, the use of the 17D-204 vaccine strain requires only BSL-2 containment, with or without vaccination, permitting pharmaceutical research to be conducted in facilities lacking high level biological containment, and presenting a reduced risk to personnel in the event of an accidental exposure. Although wt virulent YFV is not a select agent, many BSL3+ labs are registered for select agent work, so scheduling work with wt virulent YFV in these facilities could be problematic as well as requiring the YFV scientist to be unnecessarily cleared for select agent work.

The AST of 17D-204-infected AG129 mice increased in a dose-dependent fashion that correlated with a decreasing challenge dose. These data agree with two studies of YFV in AG129 mice where footpad inoculation with 10^4 PFU 17D-204 resulted in an AST of 10.1 days (Meier et al. [15]) and footpad inoculation with 10^3 PFU resulted in an AST of 18.1 days (Lee & Lobigs [18]). Our own experience with footpad inoculation of 17D-204 in AG129 mice mirrored these findings; however, i.p. inoculation yielded similar results.

In this study, AG129 mice succumbed to neurotropic disease as evidenced by characteristic clinical signs (paresis and hind limb paralysis) and high viral load in the brain at time of sacrifice. These findings agree with those previously made regarding the development of encephalitic disease in mice after challenge with either the Asibi or 17D-204. Unlike previous studies, we observed that other organs (spleen, liver, and kidney) of AG129 mice infected with 17D-204 contained significant viral loads in the early stages of infection. AG129 mice following i.p. inoculation with 10^5 PFU of 17D-204 were found to have mean titers as high as $4.96 \pm 0.01 \log_{10}$ genomic copies/gram in liver tissues by day 3, much higher than those previously reported by Lee and Lobigs [18]. This disparity may be a result of the different routes of inoculation used (s.c. versus i.p.) and/or differences in the challenge doses used (10^3 PFU versus 10^5 PFU).

The AG129-DENV model originally described by Johnson and Roehrig [16] was shown to be an effective model for studying primary DENV infection and vaccine challenge. Since then, variations of the AG129-DENV model have been used to study DENV pathogenesis and immunology [20]. In this study we have used the AG129 mouse to provide a similar model of YF for evaluating therapeutics and studying the immunopathology associated with YF infection. Using the RodentMAP® v2.0 platform we were able to construct a comprehensive inflammatory profile of 17D-204 infection of AG129 mice. Of the three tissue types analyzed, concentrations of analytes associated with inflammation showed the most fluctuation in the brain, which is not surprising since 17D-204 infection of AG129 mice results in a primarily neurotropic infection. Significant changes in inflammatory analytes were also seen in liver and serum and not surprisingly, these changes corresponded with peak viral titers in these tissues early in infection. A second peak in inflammatory analytes occurred in the liver, and to a lesser extent, in the serum at day 11 p.i., in the absence of significant virus in these two tissues. This biphasic response was associated with the appearance of morbidity in infected animals (day 9 p.i.), similar to human YFV infections.

Yellow fever is the prototypical hemorrhagic fever and in humans, YF infection is characterized by a systemic inflammation response syndrome (SIRS) that closely resembles septic shock and is suspected to contribute to multi-organ failure and death [21]. The activation of monocytes/macrophages and neutrophils, with the subsequent release of proinflammatory mediators such as tumor necrosis factor alpha (TNF- α), interleukin-2 (IL-2), IL-6, and MCP-1, are thought to play key roles in the pathogenesis of sepsis [21, 22].

Granulocyte activation also results in the release of platelet-activating factor, proteases, and leukotrienes, which may modify endothelial integrity, especially in the presence of proinflammatory cytokines, causing capillary leak [23]. To date there have been few studies investigating the inflammatory response following infection of AG129 with YFV. Meier et al. [15] reported that 17D-204 produced no changes in serum levels of MCP-1 or IL-6 in A129 mice (lacking α/β IFN receptors, intact γ IFN receptors) while infection with the Asibi caused elevated serum concentrations of both pro-inflammatory mediators that peaked by day 4 p.i. Our data indicate that infection of AG129 mice with 17D-204 caused significant increases in MCP-1 concentrations in all three tissue types tested; liver and serum MCP-1 concentrations peaked on day 3 p.i., while levels in the brain rose consistently throughout infection, peaking at day 15 p.i.. Only brain tissue homogenates showed a significant increase in IL-6 concentrations at more than one time point while liver tissue demonstrated a significantly elevated IL-6 concentration at a single time point (day 15 p.i.); IL-6 concentrations in liver tissue did increase early in infection but these increases were less than 4-fold compared to mock-infected animals. Furthermore, concentrations of IFN- γ rose significantly during infection in all tissues assayed (serum, brain, and liver). Brain and liver tissues also demonstrated significantly increased concentrations of TNF- α and IL-2. Although there are no data available in the literature regarding TNF- α response in AG129 mice following YFV infection, studies of AG129 mice infected with DENV indicate increased TNF- α levels in serum following viral challenge. Schul et al. [24] showed that serum TNF- α levels of mice infected with DENV increased 3 to 5 days p.i.; MCP-1 and IL-6 levels following DENV infection were also elevated between days 3 and 5 p.i. at levels comparable to those recorded in our AG129/17D-204 model. Similarly, studies by Shrestha et al. [25] and Stein et al. [26] reported increased TNF- α levels at day 3 p.i. in an AG129 model of dengue hemorrhagic fever/dengue shock syndrome.

Severe human YF is biphasic. Symptoms begin abruptly, lasting about 3 days, and corresponding to the time of viremia. Following defervescence, symptoms abate for about 24 h. Symptoms then reappear and severe YF ensues, resulting in a case fatality rate of 20–50% in the absence of further viral replication [28]. This course of infection is very similar to dengue disease and dengue hemorrhagic fever. Severe dengue disease, if it occurs, occurs after the period of defervescence, and has been associated with a “cytokine storm” that can result in death [27]. It is attractive to hypothesize that fatal YF infection is due to a similar late cytokine response [21, 22]. If confirmed, our observations that many cytokines peak late in the 17D-204 infection of AG129 mice – in the absence of virus – might indicate that this model system might accurately recapitulate human liver infection. This biphasic aspect of the 17D-204/AG129 mouse model deserves further investigation. Because of the short AST (6.4 days) of Asibi-infected AG129 mice, the 17D-204/AG129 mouse-infection model is a more appropriate model to investigate the biphasic aspects of YF disease.

Although infection of AG129 mice with 17D-204 results in a lethal neurotropic infection, its value as a therapeutic model for yellow fever should not be dismissed. The AG129/17D-204 model is characterized by viral replication in both central nervous system and hepatic tissues and generates significant increases in inflammatory cytokines associated with fatal YF disease. Furthermore, this model utilizes the YF vaccine strain, thus providing researchers with a model of YF that can be used to evaluate antiviral or immunomodulatory-based therapies under reduced containment (BSL-2) compared to models employing the wt virulent Asibi strain or variants thereof. In addition to its utility as a therapeutic model, the AG129/17D-204 model might also be useful in investigating the pathology associated with YF vaccine-associated neurotropic disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Human yellow fever virus infection has no good mouse model.
- We developed an adult mouse model using AG129 mice and 17D-204 vaccine.
- AG129 mice are susceptible to 17D-204 in a dose-dependent manner.
- Virus infects both brain and liver.
- Biomarkers of inflammation suggest a biphasic liver infection similar to humans.

Figure 1.

Survival of AG129 mice following i.p. challenge with 17D-204. Doses used: 1×10^6 PFU/mouse ($\diamond$); 1×10^5 PFU/mouse ($\square$); and 1×10^4 PFU/mouse ($\triangle$).

Figure 2. Scatterplot and means of virus titers expressed as genomic copies/gram (or ml) in various tissues of AG129 mice post-17D-204 challenge. Panels: a=serum; b=spleen; c=brain; d=liver; and e=kidney. Data are combined from two experiments.

Figure 3. AG129 mouse cytokine response compared to tissue virus titers. Panels: a=brain, b=liver, c=serum. Mean tissue viral titer (Δ); IFN- γ concentration (○).

Table 1

Average survival time (AST) of AG129 mice infected with 17D-204

Challenge Dose (log₁₀ PFU)	Route	% Mortality (survivors/total)	AST (days) ± SD
6	i.p.	100% (0/5)	12.0 ± 3.6
5	i.p.	100% (0/9)	15.2 ± 3.3
4	i.p.	100% (0/5)	17.2 ± 1.1
5	s.c.	100% (0/10)	16.8 ± 2.1
4	s.c.	70% (3/10)	20.8 ± 4.6

Table 2

AG129 analytes associated with inflammation or tissue damage undergoing 4-fold or greater change in concentration as a result of 17D-204 infection

Pro-inflammatory		Serum	Liver	Brain
CD40L	CD40 Ligand	↑		↑
GMCSF	Granulocyte-Macrophage Colony-Stimulating Factor			↑
KC/GRO	Growth-Regulated Alpha Protein		↑	↑
IFN γ	Interferon gamma	↑	↑	↑
IP-10	IFN γ Induced Protein 10	↑	↑	↑
IL-1 α	Interleukin-1 alpha	↓		↑
IL-1 β	Interleukin-1 beta			↓
IL-2	Interleukin-2		↑	↑
IL-6	Interleukin-6			↑
IL-11	Interleukin-11			↑
IL-12p70	Interleukin-12 subunit p70		↑	
MIP-1 β	CCL-4/Macrophage Inflammatory Protein-1 beta	↑	↑	↑
MIP-1 γ	CCL-9/Macrophage Inflammatory Protein-1 gamma			↑
MIP-2	CXCL-2/Macrophage Inflammatory Protein-2			↑
MIP-3 β	Macrophage Inflammatory Protein-3 beta			↓
MCP-1	CCL-2/Macrophage Chemotactic Protein-1	↑	↑	↑
MCP-3	CCL-7/Macrophage Chemotactic Protein-3	↑	↑	↑
MCP-5	CCL-12/Macrophage Chemotactic Protein-5	↑	↑	↑
OSM	Oncostatin-M	↑	↑	↑
RANTES	CCL-5/Regulated upon Activation, Normal T-cell Expressed, and Secreted		↑	↑
TNF α	Tumor Necrosis Factor alpha		↑	↑
Anti-inflammatory				
IL-4	Interleukin-4	↑		
IL-10	Interleukin-10			↓
Tissue Damage				
Fibrinogen	Fibrinogen		↓	
IL-7	Interleukin-7	↑	↑	↑
MMP-9	Matrix Metalloproteinase 9		↑	↑
Myoglobin	Myoglobin	↓	↑	
MPO	Myeloperoxidase			↑
SGOT	AST/Serum Glutamic Oxaloacetic Transaminase			↑
TPO	Thrombopoietin			↓