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Zika Virus Infection in Syrian Golden Hamsters and Strain 13 Guinea Pigs

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Abstract. To evaluate potential immunocompetent small animal models of Zika virus (ZIKV) infection, we inoculated Syrian golden hamsters (subcutaneously or intraperitoneally) and strain 13 guinea pigs (intraperitoneally) with Senegalese ZIKV strain ArD 41525 or Philippines ZIKV strain CPC-0740. We did not detect viremia in hamsters inoculated subcutaneously with either virus strain, although some hamsters developed virus neutralizing antibodies. However, we detected statistically significant higher viremias ($P = 0.0285$) and a higher median neutralization titer ($P = 0.0163$) in hamsters inoculated intraperitoneally with strain ArD 41525 compared with strain CPC-0740. Furthermore, some hamsters inoculated with strain ArD 41525 displayed mild signs of disease. By contrast, strain 13 guinea pigs inoculated intraperitoneally with either strain did not have detectable viremias and less than half developed virus neutralizing antibodies. Our results support the use of the Syrian golden hamster intraperitoneal model to explore phenotypic variation between ZIKV strains.

Zika virus (ZIKV) is a member of the Spondweni serogroup, genus *Flavivirus*, family *Flaviviridae*.[1] Although ZIKV is primarily transmitted by the bite of a mosquito,[2,3] sexual transmission has also been reported.[4–6] Most of the human ZIKV infections are asymptomatic, but severe clinical manifestations have been reported in a subset of infections including congenital birth defects.[7] In symptomatic cases, commonly reported signs and symptoms include rash, fever, arthralgia, myalgia, headache, conjunctivitis, edema, pruritus, and fatigue.[8] To date, both rodents and nonhuman primates have been used to model ZIKV infection.[7,9,10] However, most of the animal work to date has involved the use of immunocompromised mice which do not recapitulate human clinical infection, whereas non-murine immunocompetent rodent models have thus far been minimally investigated. Syrian golden hamsters[11] have been used for decades to study the pathogenesis of multiple arboviruses in several families, including *Flaviviridae*.[12,13] Similarly, strain 13 guinea pigs[14] have been used to study the pathogenesis of number of emerging highly pathogenic viruses.[13,15] Therefore, we performed pilot studies to investigate the potential for immunocompetent Syrian golden hamsters and strain 13 guinea pigs to model ZIKV infection using both African and Asian lineage strains.[16] Herein, we describe a hamster intraperitoneal model to explore phenotypic variation between ZIKV strains and report the results of ZIKV infection in strain 13 guinea pigs.

African lineage strain ArD 41525 was originally isolated from a pool of *Aedes africanus* mosquitoes collected in eastern Senegal in 1984 (passage history: AP61#1, C6/36#1, Vero #3; Genbank Accession no.: KU955591)[17]; whereas Asian lineage strain CPC-0740 was originally isolated in 2012 from the sera of a human patient in the Philippines (passage history: *Toxorhynchites splendens* #1, C6/36#1, Vero #2; KU681082).[18] These strains were selected because of their low passage histories, intact N-linked glycosylation sites,[16] and the absence of *Mycoplasma* spp. as confirmed by the deep sequencing of virus challenge stocks. Viruses were titered by plaque assay as previously described.[4] Plaques were counted and the results were reported as the number of plaque forming units (PFU)/mL, with a lower limit of detection of 1.0 $\log_{10}$ PFU/mL. Plaque reduction neutralization tests (PRNTs) were performed to determine post-exposure immune responses at day 21 post-inoculation (PI) as previously described.[4] Titers were calculated and expressed as the reciprocal of serum dilution yielding a > 80% reduction in the number of plaques, with a lower limit of 1:20 $PRNT_{80}$. Hamsters and guinea pigs expressing a titer of at least 1:20 were considered to have seroconverted.

Research was conducted at an AAALAC accredited institution under an IACUC-approved animal use protocol in compliance with the Animal Welfare Act, Public Health Service Policy, the Guide for the Care and Use of Laboratory Animals, and other federal statutes relating to experiments involving animals. Adult (> 100 g, 7–8 weeks) female Syrian golden hamsters (Envigo Animal Health, Haslett, MI) were socially housed in individually ventilated cage racks (Allentown Inc., Allentown, NJ), one group of three animals per cage. Mean body weight at study initiation was 120 g ± 9 g (1 SD). Adult female strain 13 guinea pigs from the USAMRIID colony, aged 7–9 months, were socially housed in stainless steel guinea pig racks (Allentown Inc), one group of three animals per cage. Mean weight at study start was 905.2 g ± 66.6 g (1 SD). Before the initiation of experiments, all animals were implanted with BioMedic IPTT-300 microchip transponders (BioMedic Data Systems Inc., Seaford, DE) in the subcutaneous space above the shoulder blades as a low-stress method to monitor daily body temperature. All animals were free of *Helicobacter* spp., *Lawsonia intracellularis, Clostridium piliforme*, Sendai virus, pneumonia virus of mice, lymphocytic choriomeningitis virus, reovirus (1, 2 and 3), mouse adenovirus (1 and 2), and simian virus 5 as determined using a combination of serological and

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molecular screening techniques (i.e., multiplex fluorescent immunoassay, indirect fluorescent antibody and/or polymerase chain reaction).[19] Our challenge dose (i.e., 5.4 $\log_{10}$ PFU/mL) aimed to mimic a moderate-to-high ZIKV exposure.[20] In our first hamster experiment, 24 hamsters, three per group, were inoculated subcutaneously with 5.4 $\log_{10}$ PFU/mL of ZIKV strain ArD 41525 (N = 12) or CPC-0740 (N = 12). Hamsters were euthanized on day 21 PI. In our second hamster experiment, 36 hamsters were intraperitoneally inoculated with 5.4 $\log_{10}$ PFU/mL of ZIKV strain ArD 41525 (N = 18) or CPC-0740 (N = 18). On days 1 and 2 PI, three hamsters inoculated with either virus strain were bled and euthanized. The remaining 24 hamsters (12 for each strain) were euthanized on day 21 PI. We also inoculated 18 guinea pigs, three per group, intraperitoneally with 5.4 $\log_{10}$ PFU/mL of ZIKV strain ArD 41525 (N = 9) or CPC-0740 (N = 9). Guinea pigs were euthanized on day 21 PI.

Hamsters and guinea pigs were observed twice daily, with temperature and weight taken once per day. Morbidity was assessed by documenting observed clinical signs of disease. Those signs of disease evaluated included abnormal behavior, weight loss, piloerection, dehydration, orbital exudates, hyperthermia (body temperature > 38.9°C), scratching, overall activity level, and response to stimuli. Blood collections were drawn from the anterior vena cava of anesthetized animals (ketamine-xylazine-acepromazine, administered intramuscularly). A staggered blood collection schedule was used to allow a recovery period for each group of animals between sample collection points. Hamsters were bled on days 1–12 PI, three animals per day, with each hamster being bled three times during this time period. Guinea pigs were also bled on days 1–12 PI, three guinea pigs bled each day, with each guinea pig being bled four times during this time period. Terminal blood collections were made on day 21 PI for both hamsters and guinea pigs before euthanasia. At euthanasia, all hamsters and guinea pigs were perfused under deep anesthesia with 0.9% sterile saline by injecting the solution directly into the left ventricle until fluid exiting the dissected jugular vein was observed to be free of blood (e.g., clear). After perfusion, all animals were necropsied and the brain, eyes, lungs, heart, liver, spleen, mesentery, kidneys, bladder, ovaries, and uterus were collected. All tissues were immersion-fixed in 10% neutral buffered formalin for at least 48 hours. Tissues were then trimmed and processed according to standard protocols.[21] All tissues were evaluated by a board certified veterinary pathologist for the presence of necrosis, increased leukocyte counts, indicators of cellular damage, and abnormal organ architecture.

Statistical analyses were performed in SAS version 9.4 (SAS Institute, Cary, NC, 2013). Viremia and $PRNT_{80}$ data were analyzed for statistically significant differences between groups of animals exposed to each strain using two-sided Wilcoxon rank-sum tests with a stepdown Bonferroni adjustment for multiple comparisons. Values below each assay's lower limit of detection were set to a value equal to the lower limit of detection divided by the square root of 2 (lower limit of detection/$\sqrt{2}$). Because of the geometric progression of the $PRNT_{80}$ results, $\log_{10}$ transformations were applied. Despite transformation, data were not normally distributed and nonparametric tests were used for analyses.

Viremia was not detected in hamsters inoculated subcutaneously; however we did detect virus neutralizing antibodies (Table 1). Mild signs of disease were observed in a few hamsters. Although not statistically significant (P = 0.0516), hamsters inoculated with strain ArD 41525 mounted a higher median virus neutralizing antibody titer (1:160, iterquartile range [IQR] 280), than those hamsters inoculated with strain CPC-0740 (1:40, IQR 65.86) by day 21 PI. No histopathological findings consistent with ZIKV infection were observed. Work elsewhere recently reported hamsters inoculated subcutaneously with a similar challenge dose of Asian lineage strain FSS13025 did not display detectable viremia or seroconversion.[22] By contrast, most of the hamsters inoculated subcutaneously in our study seroconverted (Table 1).

We detected viremia in hamsters inoculated intraperitoneally with strain ArD 41525 on day 1 and 2 PI (P = 0.0285), with some hamsters displaying mild signs of disease on days 1–4 PI (Table 2). However, viremia was not detected in hamsters inoculated intraperitoneally with strain CPC-0740 (Table 2). We did not observe weight loss in hamsters inoculated with either strain, and histopathological findings consistent with ZIKV infection were not observed. All hamsters seroconverted by day 21 PI (Table 2); those hamsters inoculated with strain ArD 41525 displayed a higher median virus neutralizing antibody titer (1:160, IQR 80), than those inoculated with strain CPC-0740

TABLE 1

Observed signs of disease and virus neutralizing antibody response in Syrian golden hamsters inoculated subcutaneously with Zika virus strain ArD 41525 or CPC-0740

Strain ArD 41525			Strain CPC-0740		
Hamster	Signs of disease (DPI)	Antibody response, ($PRNT_{80}$)*, 21 DPI	Hamster	Signs of disease (DPI)	Antibody response, ($PRNT_{80}$)*, 21 DPI
1C	–	–	1D	–	–
2C	–	1:320	2D	–	–
3C	–	1:160	3D	–	1:40
4C	–	1:20	4D	–	1:20
5C	–	NR	5D	–	1:80
6C	L (1)	1:320	6D	–	1:40
7C	L (1)	1:40	7D	–	1:80
8C	–	1:80	8D	–	1:160
9C	–	1:320	9D	–	1:40
10C	–	1:160	10D	–	–
11C	–	1:320	11D	–	–
12C	–	1:40	12D	–	1:80

DPI = day post-inoculation; L = lethargy; NR = not run – the hamster was euthanized following an adverse event unrelated to this study; PRNT = plaque reduction neutralization tests. (–) No observed signs of disease or the absence of a detectable virus neutralizing antibody response.

* Limit of detection, 1:20.

TABLE 2

Detected viremia, observed signs of disease and virus neutralizing antibody response in Syrian golden hamsters inoculated intraperitoneally with Zika virus strain ArD 41525 or CPC-0740

Strain ArD 41525				Strain CPC-0740			
Hamster	Viremia, Log_{10} PFU/mL* (DPI)	Signs of disease (DPI)	Antibody response ($PRNT_{80}$)†, 21 DPI	Hamster	Viremia, Log_{10} PFU/mL* (DPI)	Signs of disease (DPI)	Antibody response ($PRNT_{80}$)†, 21 DPI
1A	4.7 (1)	F (1–3)	1:320	1B	–	–	1:320
2A	3.7 (1)	F (1–3)	1:160	2B	–	–	1:160
3A	–	F (1–3)	1:160	3B	–	R (1)	1:80
13A‡	2.9 (1)	–	NR	13B‡	–	–	NR
14A‡	1.7 (1)	–	NR	14B‡	–	–	NR
15A‡	3.0 (1)	–	NR	15B‡	–	–	NR
4A	2.0 (2)	F (1–3), L (4)	1:160	4B	–	–	1:80
5A	2.9 (2)	F (1–3)	1:160	5B	–	–	1:80
6A	–	F (1–3)	1:320	6B	–	–	1:80
16A§	1.5 (2)	–	NR	16B§	–	–	NR
17A§	1.5 (2)	–	NR	17B§	–	–	NR
18A§	1.7 (2)	–	NR	18B§	–	–	NR
7A	–	F (1–3), T (1)	1:160	7B	–	–	1:160
8A	–	F (1–3), T (1)	1:80	8B	–	R (2)	1:40
9A	–	F (1–3)	1:160	9B	–	–	1:80
10A	–	–	1:320	10B	–	–	1:160
11A	–	–	1:160	11B	–	–	1:160
12A	–	L (4)	1:160	12B	–	–	–

DPI = day post-inoculation; F = fighting; L = lethargy; NR = not run – these hamsters were euthanized on either days 1 or 2 post-inoculation; PFU = plaque forming units; PRNT = plaque reduction neutralization tests; R = ruffled fur; T = elevated temperature (+0.5°C). (–) No detectable viremia, no observed signs of disease or the absence of a detectable virus neutralizing antibody response.

* Limit of detection, 1.0 log_{10} PFU/mL.

† Limit of detection, 1:20.

‡ Euthanized 1 day post-inoculation.

§ Euthanized 2 days post-inoculation.

(1:80, IQR 80) ($P = 0.0163$). These results demonstrate phenotypic variation between these two strains. Such in vivo phenotypic variation between African and Asian lineage strains has been reported elsewhere.[23–27]

None of the strain 13 guinea pigs inoculated intraperitoneally with either strain had detectable viremia, and no signs of disease were observed. Although virus neutralizing antibodies were observed in some strain 13 guinea pigs, less than half had virus neutralizing antibody titers greater than 1:20 (Table 3). We did not observe any histopathological findings consistent with ZIKV infection. Early ZIKV characterization involving the MR 766 strain in guinea pigs (strain not reported) also resulted in inconsistent results.[28] In contrast to our results, recent work demonstrated that Hartley guinea pigs could be used to model ZIKV infection after subcutaneous inoculation with strain PRVABC59.[29] As we used ZIKV strains from both African and Asian lineages in our study, the differences observed between both studies may be guinea pig strain specific and/or a result in differences in major histocompatibility complex Class I molecules.[30]

In conclusion, we demonstrated phenotypic variation between two ZIKV strains (ArD 41525 and CPC-0740) using a Syrian golden hamster intraperitoneal model of ZIKV infection. This immunocompetent rodent model may be useful for rapidly assessing differences in virulence and immunologic response to infection between ZIKV strains.

TABLE 3

Virus neutralizing antibody response in strain 13 guinea pigs inoculated intraperitoneally with Zika virus strains ArD 41525 or CPC-0740

Strain ArD 41525		Strain CPC-0740	
Guinea pig	Antibody response ($PRNT_{80}$)*, 21 DPI	Guinea pig	Antibody response ($PRNT_{80}$)*, 21 DPI
1E	–	1F	1:40
2E	–	2F	–
3E	1:80	3F	–
4E	–	4F	–
5E	1:80	5F	1:80
6E	1:40	6F	1:80
7E	1:40	7F	1:80
8E	–	8F	–
9E	NR	9F	–

DPI = day post-inoculation; NR = not run; PRNT = plaque reduction neutralization tests. (–) Absence of a detectable virus neutralizing antibody response.

* Limit of detection, 1:20.

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