Infectious Diseases: Research and Treatment

SHORT REPORT

In Vivo Replication and Pathogenesis of Vesicular Stomatitis Virus Recombinant M40 Containing Ebola Virus L-Domain Sequences

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Abstract: The M40 VSV recombinant was engineered to contain overlapping PTAP and PPxY L-domain motifs and flanking residues from the VP40 protein of Ebola virus. Replication of M40 in cell culture is virtually indistinguishable from that of control viruses. However, the presence of the Ebola PTAP motif in the M40 recombinant enabled this virus to interact with and recruit host Tsg101, which was packaged into M40 virions. In this brief report, we compared replication and the pathogenic profiles of M40 and the parental virus M51R in mice to determine whether the presence of the Ebola L-domains and flanking residues altered in vivo characteristics of the virus. Overall, the in vivo characteristics of M40 were similar to those of the parental M51R virus, indicating that the Ebola sequences did not alter pathogenesis of VSV in this small animal model of infection.

Keywords: VSV, Ebola, recombinant virus, budding, L-domain, pathogenesis, animal model

Infectious Diseases: Research and Treatment 2012:5 59–64

doi: 10.4137/IDRT.S10652

This article is available from http://www.la-press.com.

Introduction

Vesicular stomatitis virus (VSV) is the prototypic member of the Rhabdoviridae family of negative-sense RNA viruses. VSV is an arthropod-borne virus that causes sporadic outbreaks of disease in the United States in equine and bovine species, often characterized by the appearance of vesicular lesions resembling those induced by the foot-and-mouth disease virus.[1–4] For many years, VSV has served as a surrogate virus for the expression of a heterologous virus or host proteins and/or sequences to better understand their biological function in the context of a virus infection, or as a vector system for vaccine development or targeting tumor cells.[5–11] For example, we have used VSV as a surrogate virus to investigate the functions of late (L) budding domain motifs and flanking sequences from the VP40 matrix protein of Ebola virus.[12] Viral L-domain motifs have been well described as important components conserved within RNA virus matrix proteins that mediate recruitment of specific host proteins to facilitate efficient virus egress and dissemination.[13–15] RNA viruses with L-domain containing matrix proteins include retroviruses, arenaviruses, rhabdoviruses, paramyxoviruses, henipaviruses, and filoviruses. L-domains consist of core consensus amino acid motifs such as PPxY, P(T/S)AP, YxxL, or FPIV (x = any amino acid), which dictate the cellular interacting partner. Importantly, PPxY motifs interact with WW-domain-containing proteins such as host E3 ubiquitin ligases (eg, neuronal precursor cell-expressed developmentally down-regulated 4; Nedd4), while P(T/S)AP motifs interact with tumor suppressor gene 101 (*Tsg*101), a component of the cell vacuolar protein sorting (vps) machinery.

Reverse-genetics techniques were used to engineer and recover a VSV recombinant termed M40, which expressed the L-domains and flanking sequences from Ebola virus VP40 (RVILPTAPPEYMEAI) in place of that normally present in the M protein of VSV (LGIAPPPYEEDT).[12] The M40 recombinant provided us with a powerful tool to study the functional role of the Ebola L-domain region in the context of a virus infection under BSL-2 conditions, without the technical challenges and limitations associated with experiments involving live Ebola virus.[12]

Results from previous studies demonstrated that the Ebola VP40 L-domain region was indeed functional in mediating virus egress in the context of a VSV background.[12] For example, the M40 recombinant virus replicated to titers that were essentially identical to those of parental VSV controls, and mutations that disrupted the Ebola L-domain sequences resulted in VSV recombinants that were defective in host protein recruitment and subsequent egress from infected cells.[12] In addition, expression of the Ebola sequences did not result in any change in plaque size or in virus morphology as determined by electron microscopy.[12]

Although the in vitro cell culture growth properties of the M40 recombinant virus were similar in general to those of parental VSV strains, the unique overlapping nature of the Ebola PTAP and PPxY L-domains did result in differences in the dependence on specific host proteins for efficient budding, as compared to that of a single PPPY motif of VSV.[12] In this report, we compared levels of virus replication and pathogenic phenotypes in mice infected with either recombinant M40 or a control virus of parental M51R. Despite small differences in the levels of virus replication in the lungs and brains of infected mice, and in the kinetics of animals that eventually succumbed to infection, overall findings indicate that replication and pathogenic profiles of recombinant M40 and parental M51R in vivo were comparable.

Methods

Viruses

VSV-M51R and recombinant M40 have been described previously Figure 1.[12,16] M51R contains an arginine at amino acid position 51 within the M protein that disrupts the host shutoff function of the M protein. Recombinant M40 contains the overlapping L-domains and flanking residues from Ebola VP40 protein inserted in place of the PPPY L-domain and

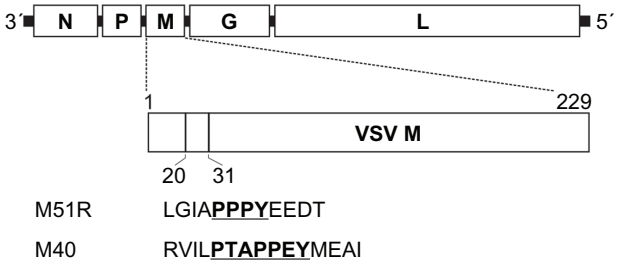

Figure 1. Diagram of the VSV genome showing an expansion of the *M* gene (229 amino acids).
Notes: The amino acid sequence of M from position 20–31 is highlighted for M51R and M40 viruses. Amino acids comprising the core L-domain motifs are underlined and in bold type.

flanking residues of VSV M protein.[12] The M40 recombinant was generated using the M51R parental background. Both viruses were propagated in BHK-21 cell and titrated by standard plaque assay.

Pathogenicity in mice

Groups of eight 6-week-old female BALB/c mice were mock-infected with PBS, or infected intranasally with 10^7 pfu/mL of M51R or M40 as described previously.[17] Body weight measurements were recorded on a daily basis over a two week period while survival was monitored and plotted. At 2 days post-infection, three mice were sacrificed, and viral titers were determined from lung and brain homogenates of sacrificed animals using a standard plaque assay on BHK-21 cells.[12]

Statistical analyses

The data shown in Figure 2 were analyzed using a 1-way ANOVA, and data shown in Figure 3 were analyzed using a *t*-test. *P*-values < 0.05 were considered to be significant.

Results and Discussion

VSV M51R and recombinant M40

The amino acids comprising the L-domain region within the parental M51R virus and recombinant M40 are shown in Figure 1. In contrast to the single PPPY L-domain motif within the M protein of M51R, the M40 recombinant was engineered to possess both the PTAP and overlapping PPEY motif from the VP40 protein of Ebola virus. The presence of the PTAP motif enables the M40 virus to recruit host protein Tsg101, which contributes to efficient budding of this recombinant virus.[12] Thus, it was of interest to determine whether the presence of the PTAP motif and the Ebola sequences in general provided any replication advantage or modification of pathogenesis for the M40 recombinant in a mouse model of infection, compared to that of control virus M51R Fig. 1.

Pathogenicity of recombinant M40 in mice

Groups of eight 6-week old BALB/c mice were either mock-infected, or infected with recombinant M40 or the M51R control virus, and mice were assessed for weight loss, survival, and replication in lung and brain tissue. Mice were inoculated with 10^7 pfu of the indicated virus and body weight was recorded on a daily basis for a two-week period (Fig. 2). Mice infected with the parental M51R virus showed a steady progression of weight loss from day 2 through day 8 (Fig. 2). A statistically significant difference in weight loss was noted on day 6 post-infection (p.i.) between M51R and PBS control ($P = 0.0497$). By day 9 p.i., all of the mice infected with M51R had succumbed to the infection. In contrast, mock-infected animals (PBS) maintained a healthy ($<$20% weight loss) body weight over the entire two-week period (Fig. 2). On average, mice infected with the M40 recombinant virus lost approximately 20% of their body weight from days 3–5 (Fig. 2). From days 6–14 p.i., the two surviving mice infected with the M40 recombinant gradually gained weight and returned to their approximate starting body weight (Fig. 2).

In correlation with body weight measurements, all mock-infected control animals survived for the 14-day period, whereas by day 9 p.i., all animals infected with the M51R virus succumbed to the infection (Fig. 2). Interestingly, several mice infected with the M40 recombinant virus succumbed more quickly to the infection compared to the rate of survival for animals infected with M51R (Fig. 2). For example, 3 of the 5 animals infected with the M40 recombinant succumbed to the infection by day 6 p.i., compared to only 1 of 5 animals infected with M51R (Fig. 2). However, eventually all 5 mice infected with M51R succumbed to the infection by day 9 p.i., whereas 2 of the 5 mice infected with the M40 recombinant survived through day 14 p.i. (Fig. 2). Thus, while the survival kinetics varied slightly between animals infected with M51R versus those infected with the M40 recombinant (not statistically significant), the presence of the Ebola virus VP40 sequences within the *M* gene of VSV did not significantly alter the pathogenic phenotype of the virus in this small animal model of infection. These in vivo findings correlate well with those described previously using cell culture to assess and compare infection and replication of M51R and M40 viruses.[12]

Replication of recombinant M40 in mice

In addition to monitoring weight loss and survival, we also determined the level of virus replication in both lung and brain homogenates from animals infected with either M51R or the M40 recombinant.

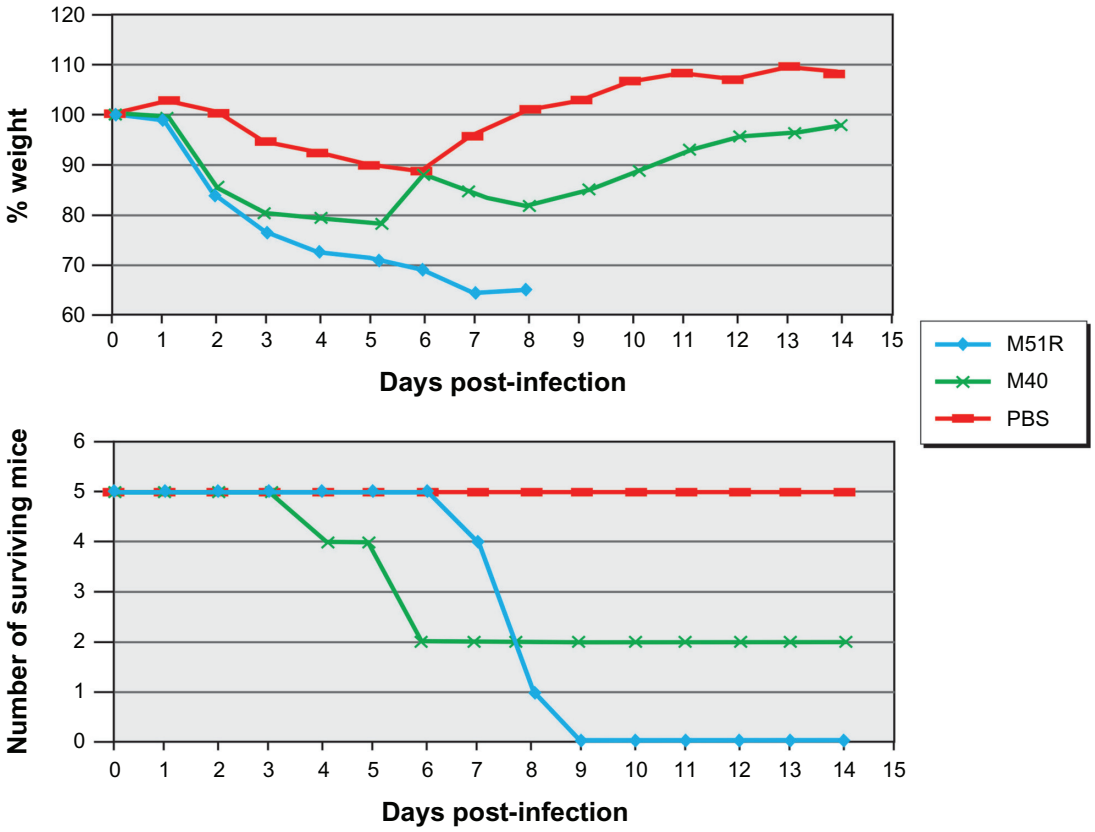

Figure 2. Graphs of weight change and survival in mice. (Top) The percentage weight change for mock-infected mice (PBS) and mice infected with either M51R or M40 viruses is shown. (Bottom) Survival curves over a two week period for mice that were mock-infected (PBS), or infected with M51R or M40 viruses are shown.
Notes: A 1-way ANOVA was performed and *P*-values were calculated for days 1–14 for both weight loss and survival. All calculated *P*-values were >0.05, except for a *P*-value of 0.0497 determined for weight loss between M51R and PBS on day 6 p.i.

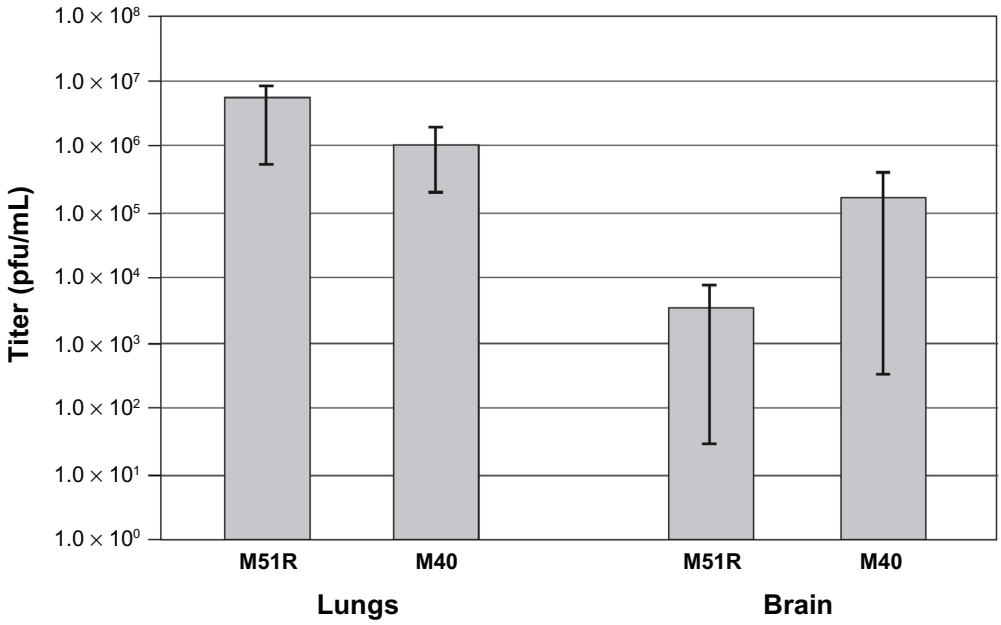

Figure 3. Replication of M51R and M40 in lung and brain tissue of infected mice.
Notes: The average viral titers (pfu/mL) from lung and brain homogenates of three mice for each group were determined using a standard plaque assay on BHK-21 cells performed in triplicate. A t-test was used to calculate *P*-values and determine the statistical significance of the reported viral titers. All calculated *P*-values were >0.05, and thus the differences in viral titers are considered to be non-significant.

Three mice from each group were euthanized on day 2 p.i. for titration of the virus in both lung and brain tissues, using a standard plaque assay on BHK-21 cells (Fig. 3). The average titer for M51R in the lung was 5.6×10^6 pfu/mL, and the average titer for M40 in the lung was 9.7×10^5 pfu/mL (Fig. 3). More variation in viral titers was observed in brain samples, where the average titer for M51R was 3.2×10^3 pfu/mL and the average titer for M40 was 1.6×10^5 pfu/mL (Fig. 3).

The ability to detect VSV replication in the lungs and brains is typical in this small animal model of infection. The replication levels of the M40 recombinant virus observed in the lungs indicates that M40 does not possess an attenuated phenotype in mice. Moreover, detection of M40 replication in the brains of infected animals also supports a pathogenic phenotype for M40 similar to that observed for the parental control M51R virus. If the Ebola L-domain sequences were providing an overall replication (budding) advantage to M40 in infected mice, then we might have expected to observe significantly higher titers for M40 in the lungs and brains, and more severe mortality rates than the M51R parental control. Statistical analysis indicated that the differences in observed titers in the lungs and brains of mice infected with M51R and M40 were not statistically significant ($P > 0.05$). We conclude that the presence of the Ebola L-domain sequences do not provide an overall replication advantage, nor do they provide enhanced pathogenic properties to the M40 virus, as indicated by this small animal model of infection.

Conclusions

Overall, the data presented here indicate that the presence of Ebola VP40 L-domain sequences expressed within the M protein of VSV did not result in a major difference in the ability of the M40 recombinant to replicate and cause disease in a mouse model of infection, compared to that of the parental M51R strain. These in vivo data correlate well with previously published findings for the replication properties of the M40 recombinant in cell culture.[12] Thus, although the presence of different L-domain motifs does dictate the identity of specific host proteins recruited for virus budding,[12] the M40 L-domain does not appear to provide any obvious replication advantage to the virus in either in vitro[12] or in vivo (as found in this report) models for VSV infection. Interestingly, a recent report suggests that L-domain motifs for VSV may function in a cell type dependent manner.[18] Thus, it is possible that the M40 recombinant may possess enhanced or reduced replication and/or pathogenic potential in a specific cell type or animal/insect host that has not yet been identified.

Acknowledgments

The authors would like to thank members of the Harty laboratory for critical comments on the manuscript, Shiho Irie for excellent technical assistance, and Deborah Argento for assistance in preparation of the figures.

Funding

Support for this study was provided in part by NIH grants AI46499 and AI090284 (R.N.H.). This study was supported in part by CIVIA, an NIAID-funded center (U19 AI62623) to investigate viral immune antagonism (A. G.-S.).

Author Contributions

Conceived and designed the experiments: T.I., E.C., A.G-S., and R.N.H. Analyzed the data: T.I., E.C., A.G-S., and R.N.H. Wrote the first draft of the manuscript: T.I. and R.N.H. Contributed to the writing of the manuscript: T.I., E.C., A.G-S., and R.N.H. Agree with manuscript results and conclusions: T.I., E.C., A.G-S., and R.N.H. Jointly developed the structure and arguments for the paper: T.I., E.C., A.G-S., and R.N.H. Made critical revisions and approved final version: T.I., E.C., A.G-S., and R.N.H. All authors reviewed and approved of the final manuscript.

Competing Interests

Author(s) disclose no potential conflicts of interest.

Disclosures and Ethics

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References

1. McCluskey BJ, Mumford EL. Vesicular stomatitis and other vesicular, erosive, and ulcerative diseases of horses. *Vet Clin North Am Equine Pract*. 2000;16(3):457–69, viii–ix.
2. Novella IS. Contributions of vesicular stomatitis virus to the understanding of RNA virus evolution. *Curr Opin Microbiol*. 2003;6(4):399–405.
3. Rodriguez LL. Emergence and re-emergence of vesicular stomatitis in the United States. *Virus Res*. 2002;85(2):211–9.
4. Bennett KE, Hopper JE, Stuart MA, West M, Drolet BS. Blood-feeding behavior of vesicular stomatitis virus infected Culicoides sonorensis (Diptera: Ceratopogonidae). *J Med Entomol*. 2008;45(5):921–6.
5. Barber GN. Vesicular stomatitis virus as an oncolytic vector. *Viral Immunol*. 2004;17(4):516–27.
6. Geisbert TW, Feldmann H. Recombinant vesicular stomatitis virus-based vaccines against Ebola and Marburg virus infections. *J Infect Dis*. 2011; 204 Suppl 3:S1075–81.
7. Li S, Locke E, Bruder J, et al. Viral vectors for malaria vaccine development. *Vaccine*. 2007;25(14):2567–74.
8. Lichty BD, Power AT, Stojdl DF, Bell JC. Vesicular stomatitis virus: re-inventing the bullet. *Trends Mol Med*. 2004;10(5):210–6.
9. McKenna PM, McGettigan JP, Pomerantz RJ, Dietzschold B, Schnell MJ. Recombinant rhabdoviruses as potential vaccines for HIV-1 and other diseases. *Curr HIV Res*. 2003;1(2):229–37.
10. Tani H. Development of viral vectors and the application for viral entry mechanisms. *Uirusu*. 2011;61(1):99–107.
11. Xing Z, Lichty BD. Use of recombinant virus-vectored tuberculosis vaccines for respiratory mucosal immunization. *Tuberculosis (Edinb)*. 2006;86(3–4): 211–7.
12. Irie T, Licata JM, Harty RN. Functional characterization of Ebola virus L-domains using VSV recombinants. *Virology*. 2005;336(2):291–8.
13. Bieniasz PD. Late budding domains and host proteins in enveloped virus release. *Virology*. 2006;344(1):55–63.
14. Chen BJ, Lamb RA. Mechanisms for enveloped virus budding: can some viruses do without an ESCRT? *Virology*. 2008;372(2):221–32.
15. Harty RN, Schmitt AP, Bouamr F, Lopez CB, Krummenacher C. Virus budding/host interactions. *Adv Virol*. 2011;2011:963192.
16. Irie T, Licata JM, McGettigan JP, Schnell MJ, Harty RN. Budding of PPxY-containing rhabdoviruses is not dependent on host proteins TGS101 and VPS4A. *J Virol*. 2004;78(6):2657–65.
17. Irie T, Carnero E, Okumura A, Garcia-Sastre A, Harty RN. Modifications of the PSAP region of the matrix protein lead to attenuation of vesicular stomatitis virus in vitro and in vivo. *J Gen Virol*. 2007;88(Pt 9):2559–67.
18. Obiang L, Raux H, Ouldali M, Blondel D, Gaudin Y. Phenotypes of vesicular stomatitis virus mutants with mutations in the PSAP motif of the matrix protein. *J Gen Virol*. 2012;93(Pt 4):857–65.