Generation of Polyclonal Rabbit Antisera Specific to the Zika Virus Capsid Protein

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ABSTRACT
Zika virus (ZIKV), a mosquito-borne flavivirus, is an emerging zoonotic pathogen closely related to Japanese encephalitis virus, West Nile virus, dengue virus, and yellow fever virus. Although ZIKV infection generally produces only mild symptoms in some infected individuals, it has recently been associated with a growing number of neurological diseases, including Guillain-Barré syndrome in ZIKV-infected adults and microcephaly in infants born to ZIKV-infected women. Like all flaviviruses, ZIKV has a plus-strand RNA genome encoding ten functional proteins (designated C, prM, E, NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). Of these ten, the C (capsid) protein is an essential structural protein required for the formation of infectious viral particles. In order to produce the antisera specifically recognizing the ZIKV C protein in this study, we expressed and purified the ZIKV C protein as a glutathione-S-transferase (GST) fusion protein in E. coli. The ZIKV C protein-coding region was PCR-amplified using the genomic RNA of ZIKV PRVABC-59, and the amplicons were cloned into the pGEX-4T-1 E. coli expression vector. GST-C fusion proteins were purified using a glutathione sepharose column. Subsequently, the GST-C fusion proteins were used for immunization with rabbits. Western blot analysis using the ZIKV-infected Vero cell lysates were performed to examine the reactivity of the antisera to the ZIKV C protein. Thus, this study provides a useful reagent for the diagnosis and understanding of the viral morphogenesis in the ZIKV-infected cells.

INTRODUCTION
ZIKV is a positive-stranded RNA virus of the family Flaviviridae, genus Flavivirus. ZIKV is spread among humans primarily through the bite of infected Aedes species mosquitoes, but it can also be transmitted from an infected mother to her child during pregnancy or by sexual contact or blood transfusion. In nature, ZIKV is probably maintained in a sylvatic cycle involving wild primates and arboreal mosquitoes. Although ZIKV infection generally causes a mild and self-limiting illness in infected individuals, it has recently been associated with a growing number of severe neurological disorders, including Guillain-Barré syndrome in adults and microcephaly in newborns. Historically, ZIKV spread from equatorial Africa and Asia to the Pacific Islands, and most recently to Latin America, and it is now a pandemic in progress [Yun et al., Genome Announcements, 2016, In press].

METHODS & RESULTS
The ~12-kDa C protein has several roles during viral replication, including the formation of a viral core, packaging of the genomic RNA, and interaction with the cellular membranes. The C protein is first translated as a membrane-bound polyprotein precursor, and the virion-associated form of the C protein is created when the transmembrane domain is cleaved by the viral serine protease NS3 with its cofactor NS2B. The C protein is highly basic, which fits the role it plays in binding to the genomic RNA in viral assembly. The C protein has multiple hydrophobic and basic domains. The hydrophobic domains allow binding to the cellular membrane, while the basic domains admit interaction with the genomic RNA. Formation of virus particles is coordinated by interactions amongst the membrane-bound C proteins, interacting with the genomic RNA, and the membrane proteins prM and E. It is suggested that this process is coordinated by the proteolytic processing in membrane-associated polyprotein precursors.

CONCLUSIONS & APPLICATIONS
Antisera has an amazing ability to produce a more robust immune system in individuals that are inoculated. In this way passive immunity to the Zika Virus can be passed on to those who are not currently able to fight off the virus. The high specificity of our antisera hopefully will create an efficient and accurate response to any Zika Virus antigens.

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