**Zika Vaccine Development: Flavivirus Foils**

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The current Zika virus outbreak has galvanized the public health community, resulting in rapid action by entities ranging from the World Health Organization to the United States government. The rapid response to Zika is perhaps the first of its kind, and it undoubtedly has been made possible by the lessons learned from the response to the 2014 Ebola virus outbreak in West Africa. However, Zika virus is not Ebola virus. As of February 2016 there were only 296 publications on "Zika" in the PubMed database, and a large number of these publications were commentaries or reviews lacking primary research data. In contrast, work had been underway for decades on the development of an Ebola virus vaccine, laying the groundwork for a rapid response in 2014. The broader community's extensive experience with Dengue virus vaccine development and with the pros and cons of different vaccine platforms has led to speculation that a Zika virus vaccine can be accelerated, potentially with clinical trials initiating by the end of 2016 [1,2]. However, there are unique attributes of Zika virus, as well as many unanswered questions about the virus, that should be considered before a potential vaccine is administered to the public.

Perhaps the best understood aspect of Zika virus is its close relation to Dengue virus and other flaviviruses. The close phylogenetic relationship between the flaviviruses has confounded diagnostic efforts as antibody elicited by viruses in this family cross-react [3,4]. Definitive diagnosis of Zika infection in individuals with previous flavivirus infection requires detection of viral RNA by PCR, which is only achievable during approximately a two week window early in infection. Efforts are underway to develop serological assays to distinguish the flavivirus infections [5], which would significantly advance our understanding of the epidemiology of Zika virus. However, the cross-reactivity of antibody elicited by Dengue and Zika is more than a diagnostic inconvenience. This relationship should also be considered in the context of the efficacy of potential vaccine candidates in the at-risk population.

**Antigenic Sin**

The principle of "original antigenic sin" was first described in 1953 by Francis et al in the context of influenza exposure [6-8]. The authors observed that successive exposure to closely related viral variants induced a preferential response to the initially encountered variant. The impact of this is that with each annual exposure to influenza virus, memory immune responses to conserved antigens will be reactivated and dominate the immune response to the current viral agent. However, an immune response to these conserved antigens may not be the effective, required response. Thus despite potentially robust antibody titers in response to infection, the immune response may be ineffective as it is not targeting the critical (non-conserved) antigen domains.

This phenomenon has been extensively studied with influenza infection, and it is considered to be an impediment to vaccine development. New technology in antibody epitope mapping led researchers to demonstrate that there are clear differences in how the antibody response of toddlers, who had no pre-existing immune response to influenza hemagglutinin, differed from that of adults who had previous exposure [9]. In this study, an antibody response targeting the entire length of HA1 and HA2 was elicited in toddlers; in contrast, vaccination of adults led to a preferential expansion of pre-existing antibody responses to HA2 and minimal responses to the less conserved HA1. While this might cause concern that adults would therefore lose their ability to respond to novel influenza antigen, the group also provided a potential solution to the problem. Inclusion of an adjuvant, in this case MF59, significantly enhanced the ability of the adults to respond to novel protein domains. While the memory B cell responses to HA2 expanded regardless of adjuvant inclusion, inclusion of adjuvant seemingly permitted the activation of naïve B cells that were specific for the less conserved protein regions [9]. A more recent study by this group

suggests that inclusion of adjuvant, in this case ISCOMATRIX™, can also enhance antibody affinity for novel antigen [10].

Dengue virus investigators are well acquainted with the principle of original antigenic sin [11-13]. As early as 1983, investigators observed that patients exposed to multiple Dengue serotypes had higher neutralizing antibody responses to the initial infecting serotype rather than to the subsequent infecting serotypes [11]. The impact of infection with multiple DENV serotypes is complicated by the issue of antibody dependent enhancement (ADE), which will be discussed in the next section, but the implications for the immune response to Zika virus infection have yet to be evaluated.

Risk of Zika virus infection is directly linked to proximity to *Aedes aegypti* and *Aedes albopictus*, the mosquito species that carry the virus. Therefore individuals at risk for infection have potentially been previously exposed to other vector-borne pathogens, including Dengue, Chikungunya, Yellow Fever, or Usutu, among others. The possibility that pre-existing antibody specific for flaviviruses like Dengue, Yellow Fever, or Usutu might preclude the development of an optimal anti-Zika adaptive immune response is a real risk, and could have significant implications for vaccine development. Less immunogenic vaccine platforms like DNA or subunit based vaccines may therefore be suboptimal in the endemic population, even if they elicit robust immune responses in flavivirus-naïve populations. It is critical that the public health community and vaccine researchers consider the target population for their vaccines, and that Phase I trials be conducted in endemic populations, to determine the impact of pre-existing immunity on the response to the vaccine. Studies dissecting the interplay between pre-existing immunity and the response to investigational vaccines would also be of significance to investigators across the spectrum of basic and applied research.

**Antibody Dependent Enhancement**

Dengue virus vaccine development has been an active area of research for years, but the virus has proven to be a complex candidate for vaccine development. There are four serotypes of Dengue virus, and it was demonstrated that primary infection with one serotype could actually enhance the pathogenicity of secondary infection with a different serotype [14,15]. Therefore a vaccine designed against serotype 2 could potentially enhance the symptoms associated with a subsequent infection with serotype 4. One proposed mechanism behind this phenomenon is ADE of infection. ADE is the phenomenon whereby antigen-specific antibodies bind the virus and target the immune complex to Fc-receptor expressing cells, which are highly permissive to Dengue virus infection, resulting in higher titers of virus [16-18]. Consequently, the antibody is actually enhancing the infectivity of Dengue virus.

To circumvent this complication, millions of dollars and countless man hours have gone into developing optimized antigen and vaccines to elicit protection against all four serotypes [19,20]. Multiple vaccines are currently in clinical trials, and investigators are exploring the relevance of preexisting immunity on vaccine mediated protection. Confounding these efforts is the observation that neutralizing antibody levels against specific serotypes of Dengue do not always predict protection, leaving investigators still seeking predictive correlates of protection [16,21-23].

Just as Zika is not Ebola virus, with a plethora of vaccine options advanced in the pipeline, neither is it Dengue virus. Zika, as far as we know, does not have multiple serotypes and we do not anticipate that ADE of Zika infection will be an issue with a future vaccine. However, there is a possibility that a vaccine developed against Zika would elicit antibodies that cross-react with Dengue virus. These antibodies could then exacerbate ADE during subsequent Dengue virus infections.

If the connection between Zika virus infection and microcephaly is demonstrated to be causative, certainly the need for a Zika vaccine is critical. However, Dengue virus infection and the disease associated with it are significantly more problematic for the vast majority of infected patients than is Zika virus infection. Infection with Dengue virus can be fatal and has a significant impact on the cost of the public health system in developing countries. The rapid distribution of a vaccine with the potential to exacerbate Dengue virus infection would be ill-advised and potentially cause more harm than good.

**Summary**

The development of a vaccine for Zika virus is unequivocally an important goal, particularly if the correlation with fetal abnormalities, as well as Guillain Barre syndrome, is proven to be causative [24]. However, when dealing with a pathogen about which so little is known, it is critical to consider the worst case scenarios and evaluate safety and efficacy in the most thorough manner possible. Zika has surprised us already with its apparent ability to be transmitted sexually and its association with microcephaly [25-27]. Methodical evaluation and open discourse regarding potential safety risks is imperative, particularly considering that women of child-bearing age and pregnant women may be primary recipients of the vaccine.

In this discussion, we have raised the question of two possible complications associated with the development of a safe and effective vaccine: one, the immunogenicity of a potential vaccine may be adequate in a flavivirus-naïve population but inadequate in an endemic population; two, vaccination with a Zika-targeting vaccine may have a negative impact on subsequent Dengue or other flavivirus infections due to ADE. In no way are we suggesting a slowing or cessation of vaccine development efforts; indeed, the fact that these complications have not been observed with the Yellow Fever vaccine suggests that such concerns may be soon put to rest. However, we do advocate for clinical studies, retrospective and prospective, to investigate the relationship between Zika pathogenesis and pre-existing flavivirus exposure. These could easily be run concurrent with vaccine development efforts. A coordinated effort with shared data between investigators and vaccine developers, in combination with evaluation of vaccine candidates in the appropriate populations with the appropriate controls, may be the most efficient route to truly accelerating the development of a safe and effective Zika virus vaccine.

**Disclaimers**

Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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