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The relative contribution of antibody and CD8$^+$ T cells to vaccine immunity against West Nile encephalitis virus

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Summary West Nile virus (WNV) is a mosquito borne, neurotropic flavivirus that causes a severe central nervous system (CNS) infection in humans and animals. Although commercial vaccines are available for horses, none is currently approved for human use. In this study, we evaluated the efficacy and mechanism of immune protection of two candidate WNV vaccines in mice. A formalin-inactivated WNV vaccine induced higher levels of specific and neutralizing antibodies compared to a DNA plasmid vaccine that produces virus-like particles. Accordingly, partial and almost complete protection against a highly stringent lethal intracranial WNV challenge were observed in mice 60 days after single dose immunization with the DNA plasmid and inactivated virus vaccines, respectively. In mice immunized with a single dose of DNA plasmid or inactivated vaccine, antigen-specific CD8$^+$ T cells were induced and contributed to protective immunity as acquired or genetic deficiencies of CD8$^+$ T cells lowered the survival rates. In contrast, in boosted animals, WNV-specific antibody titers were higher, survival rates after challenge were greater, and an absence of CD8$^+$ T cells did not appreciably affect mortality. Overall, our experiments suggest that in mice, both inactivated WNV and DNA plasmid vaccines are protective after two doses, and the specific contribution of antibody and CD8$^+$ T cells to vaccine immunity against WNV is modulated by the prime-boost strategy.

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Introduction

West Nile virus (WNV) is a mosquito borne, neurotropic, single-stranded RNA *Flavivirus*, that is closely related to other human pathogens such as Japanese encephalitis, Saint Louis encephalitis, tick-borne encephalitis, yellow fever (YFV) and Dengue viruses. Humans infected with WNV develop a febrile illness that can progress to meningitis or encephalitis or acute flaccid paralysis. The elderly and immunocompromised are at greatest risk for severe encephalitic disease. At present, treatment for WNV infection is supportive and no vaccine is approved for human use [1—3].

Studies in animal models indicate that both innate and adaptive immune responses are required to protect against primary infection by virulent strains of WNV. Type I (α/β) and type II (γ) interferon, $\gamma\delta$ T cell activation, an early neutralizing IgM response, and CD4+ and CD8+ T cells all contribute to and orchestrate control and clearance of WNV from peripheral and CNS tissues (reviewed in [4]). The induction of high-titer WNV-specific and neutralizing antibodies after infection has been assumed as a primary mechanism of control during secondary challenge. Indeed, passive transfer of immune serum, monoclonal antibodies or polyclonal antibodies protects rodents from lethal primary WNV infection [5—11].

Since its introduction into United States in 1999, several classes of candidate WNV vaccines have been developed (reviewed in [12]). Although attenuated and inactivated viral vaccines currently protect horses and birds against WNV infection, no vaccine is approved for human use. Formalin-treated WNV protects geese, hamsters, and horses from lethal experimental WNV challenge [13—18], and horses in field trials [16,19,20]. Although inactivated vaccines may be useful for immunocompromised individuals, repeated dosing may be required to induce durable protection. DNA plasmid vaccines also elicit protective immunity in animal models. Administration of plasmid DNA encoding the prM and E proteins prevented both viremia and mortality in horses, mice, and crows [18,21—23]. Plasmid based DNA vaccines induced robust helper T cell immune responses, cytokine production, and humoral immunity [21]. Vaccination with multiple doses of purified recombinant WNV E protein or domain III also elicited neutralizing antibodies and protected mice from WNV challenge [24—26]. However, in some of these studies, mice immunized with recombinant protein succumbed to high dose WNV challenge, suggesting that the efficacy of subunit vaccination may be limited by a failure to stimulate CD8+ T cell immunity. Live-attenuated WNV vaccines derived by serial passage have also been evaluated: vaccination with an attenuated Israeli isolate protected mice and geese from lethal challenge with a virulent WNV stain [27,28]. A plasmid expressing the full-length genome of an attenuated Kunjin strain of WNV was also protective in mice [29]. Chimeric WNV strains have also been developed as candidate vaccines [30,31]. A YFV-WNV chimeric vaccine induced neutralizing antibodies and T cell responses, was completely protective against lethal WNV challenge in monkeys [30], and generated robust immune responses in healthy human volunteers [32].

Many vaccines are believed to protect against infectious agents by inducing humoral and cellular immune responses. A fundamental area of vaccine research is to identify the surrogate markers of humoral and cellular immunity that predict the protective activity of a vaccine in a population that cannot be experimentally challenged. Although most vaccine platforms have been evaluated in terms of production of neutralizing antibodies and induction of T cell responses, their relative importance with respect to protection has not been studied in detail. Herein, we evaluate in detail the development of WNV-specific humoral and CD8+ T cell responses after vaccination with DNA plasmid or formalin-inactivated vaccines. By performing challenge experiments in mice that have been depleted or are genetically deficient in CD8+ T cells, we define the relative roles of antibody and CD8+ T cells in vaccine protection against WNV.

Methods

Cell culture and virus strains

Baby hamster kidney fibroblast (BHK21) cells were used to determine viral titers as described previously [5]. The lineage I WNV strain (3000.0259) was isolated in New York (2000) and passaged once in C6/36 cells to create a stock virus (2×10^8 PFU/ml) as described previously [5].

Inactivated virus and DNA plasmid WNV vaccines

A formalin-inactivated whole virion (New York 1999 strain VM2, serial #1666142A) veterinary vaccine, prepared at Fort Dodge Animal Health, was used in the present study. This vaccine is available commercially (West Nile Innovator™) and protects horses when administered as two 1 ml doses, 3—4 weeks apart [18]. This inactivated virus vaccine is not gradient purified and likely contains additional viral proteins including the non-structural proteins. For experiments in this study, different doses of the inactivated vaccine 100 μl, 10 μl, or 1 μl were tested. A DNA plasmid vaccine was prepared at Fort Dodge; it encodes for the premembrane (prM) and the envelope (E) protein of WNV New York 1999 strain resulting in the production of virus-like particles (VLP) [18]: 10 or 100 μg of DNA was used for each immunization per mouse. Both the inactivated and DNA vaccines contained a proprietary oil adjuvant, MetaStim® as well as an excipient. Placebo vaccines contained only the adjuvant and excipient.

Mice used in experiments

C57BL/6J strain (H-2^b) inbred wild type and congenic CD8α-chain$^{-/-}$ (CD8α^{tm1mak}) mice were obtained from Jackson Laboratory (Bar Harbor, Maine). All experiments were performed in the animal facilities with approval and under guidelines of the Washington University Animal Studies Committee.

Animal immunization and protection studies

Eight-week-old mice were immunized with formalin-inactivated virus or DNA plasmid WNV vaccines. In most of the studies with inactivated vaccines, animals received