



Identification of a conserved neutralizing epitope on the ASFV H240R protein by a nanobody that inhibits viral attachment

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African swine fever virus (ASFV), a highly contagious double-stranded DNA virus, causes a hemorrhagic disease with mortality approaching 100% in domestic pigs. Despite intensive efforts, effective vaccine options remain limited, highlighting the need for continuously identifying viral neutralizing epitopes. Nanobodies can effectively engage cryptic epitopes that are often inaccessible to conventional antibodies due to their small size. Here, we immunized a Bactrian camel with recombinant ASFV H240R expressed in a bacterial system, and an H240R-specific VHH phage-display library was constructed and screened, yielding a nanobody named H240R-Nb82. H240R-Nb82 efficiently neutralized distinct ASFV isolates (genotypes I, II, and their recombinants) in porcine alveolar macrophages. Mechanistically, H240R-Nb82 inhibited the early stage of ASFV infection by blocking viral attachment to host cells. Epitope mapping using truncation analysis and site-directed alanine mutagenesis showed that H240R-Nb82 binds to a linear, native virion-accessible conserved region within H240R (residues ³³GHYPFSFELK⁴²) and His34 made an important contribution to H240R-Nb82 binding. H240R-Nb82 also directly bound purified ASFV virions. Moreover, immunization of mice with the epitope-containing antigen induced sera capable of neutralizing ASFV infection in porcine alveolar macrophages, demonstrating the protective potential of this conserved determinant. Collectively, these findings define a previously unrecognized conserved neutralizing epitope on ASFV H240R and provide a new target protein for developing ASFV subunit vaccines.

African swine fever virus (ASFV) is a large, structurally complex DNA virus that causes African swine fever (1). The outbreaks have resulted in sustained and significant losses to the global swine industry (2). However, the safe and broadly effective commercial vaccines are scarcely available, a core obstacle being that the protective immune mechanisms against the virus are not fully understood (3). Previous studies have indicated that the traditional inactivated vaccines fail to provide protection, seemingly suggesting a difficulty in eliciting effective neutralizing antibodies (4).

ASFV possesses a large genome encoding approximately 150 to 200 proteins from 161 to 167 open reading frames (5). The mature virion is characterized by a complex, multilayered architecture comprising a nucleoid, core shell, inner envelope, capsid, and outer envelope (6). While this overall structural framework is established, the specific functions of most ASFV-encoded proteins, particularly those constituting the virion, remain poorly defined (7). Elucidating these roles is crucial for advancing rational strategies in ASFV vaccine and diagnostic development (8).

In general, proteins located on the outer envelope are considered particularly important for understanding viral attachment, entry, and antibody-mediated neutralization, because they are directly exposed to the extracellular environment and are readily accessible to host antibodies or other binding molecules (9). However, ASFV infectivity is not necessarily restricted to virions with a fully intact outer envelope. Previous studies have suggested that infectious ASFV particles may include virions lacking the outer envelope or particles whose outer envelope is disrupted during viral entry, and these particles can still retain infectivity (10). Therefore, under certain infection-related conditions or in specific virion states, proteins located beneath the outer envelope, particularly those constituting the viral capsid, may become exposed and accessible to neutralizing

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