Title:

An Adenovirus Vector Expressing a Single VHH-Based Neutralizing Agent (VNA) Protects Piglets from Fatal Systemic Complications Induced by Infection with Shiga Toxin Producing E. coli

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Abstract:

Infection with Shiga toxin (Stx)-producing Escherichia coli (STEC) is the most significant cause of hemolytic uremic syndrome (HUS), the leading cause of acute renal failure in children. Of the two antigenically distinct toxins, Stx1 and Stx2, Stx2-producing E. coli strains are more frequently associated with HUS than strains that produce both Stx1 and Stx2; while Stx1 alone has rarely been associated with HUS. There is no effective treatment or prophylaxis for HUS available clinically. Human monoclonal (HuMAb) against Stx2, produced by our team were shown to be highly effective when administered after bacterial challenge. The systemic administration of VHH-based neutralizing agents (VNAs) that can be economically produced and target both Shiga toxins may be a superior approach for therapies that prevent or treat STEC sequelae such as HUS. Camelids produce heavy chain-only antibodies and their antigen-binding VH domains (VHH) bind antigens without light chain domain pairing. We recently produced a panel of VHH domains that neutralize Stx1 and/or Stx2 in cell-based assays. Linking two VHHs targeting the same toxin into a single protein results in substantial improvements in neutralization potency compared to the monomer VHH components. We linked three neutralizing VHHs (one specific for each Stx and one cross-specific for both Stxs) to produce one VNA that neutralizes both Stx1 and Stx2 (VNA-Stx1/Stx2) with extreme potency. This VNA is highly protective in mice exposed to lethal doses of either Stx1 or Stx2. VNAs are highly amenable to gene therapy strategies that employ genetic delivery vehicles to promote de novo serum expression of toxin neutralizing activities. We produced a non-replicating adenovirus vector that promotes secretion of functional VNA-Stx1/Stx2 (Ad-VNA-Stx1/Stx2) from infected cells. This virus was parenterally administered to mice 4h prior to Stx2 challenge and protected all mice from fatal intoxication. Furthermore, parenteral injection of the Ad-VNA-Stx1/Stx2 about a day prior to oral STEC challenge protected 5/5 gnotobiotic (GB) piglets from Stx2-mediated neurological complications and death, whereas 0/5 untreated controls survived

the systemic complications. We are currently determining the protective efficacy of Ad-VNA-Stx1/Stx2 in relation to the time of treatment after the onset of diarrhea of STEC infected GB piglets. In conclusion, genetic delivery of VNA-Stx1/Stx2 shows excellent potential to prevent Stx-mediated fatal systemic complications.