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Prevention of the murine model of biliary atresia after live rotavirus vaccination of dams

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Abstract

Purpose: Biliary atresia (BA) is a neonatal disease that results in the obliteration of the biliary tree. The murine model of BA has been established where rhesus rotavirus (RRV) infection of newborn mice leads to an obstructive cholangiopathy. We determined whether maternal postconception rotavirus vaccination could prevent the murine model of BA.

Materials and Methods: Female mice were mated and injected intraperitoneally with one of the following materials: purified rotavirus strains RRV or Wa, high or low-dose Rotateq (Merck and Co Inc, Whitehouse Station, NJ) (a pentavalent rotavirus vaccine [PRV]), purified recombinant viral antigens of rotavirus (VP6) or influenza (NP), or saline. B-cell–deficient females also underwent postconception PRV injection.

Results: Maternal vaccination with PRV improves survival of pups infected with RRV. Serum rotavirus IgG, but not IgA, levels were increased in pups delivered from dams who received RRV, Wa, PRV, or VP6, but in the case of the Wa, PRV, and VP6 groups, these antibodies were not neutralizing. Postconception injection of high-dose PRV did not improve survival of pups born to B-cell–deficient dams.

Conclusion: Maternal vaccination against RRV can prevent the rotavirus-induced murine model of BA in newborn mouse pups.

Biliary atresia (BA) is a neonatal disease characterized by inflammation and fibrosis that result in progressive obliteration of the extrahepatic biliary tree. Worldwide, the incidence of this disease is estimated between 1 in 5000 to 18,000 live births. Biliary atresia is the most common indication for pediatric liver transplantation in the United States, accounting for 50% of these cases yearly [1].

Despite its clinical significance, the etiology of BA has not been established. In 1972, Landing [2] proposed that cholangiopathic disease including BA may be because of an external insult that leads to a progressive inflammatory process. Although it is unclear what this external factor may be, both patient-based clinical reports and basic science research suggest that viral infection may be a

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possible trigger. A viral "initiator" in the pathogenesis of BA has been supported by human studies that have identified a number of putative viruses [3-5], but the most compelling evidence exists for cytomegalovirus [4,6,7], reovirus [8], and rotavirus [9].

Murine studies involving reovirus and rotavirus, both members of the *Reoviridae* family, have supplemented our understanding of BA. The murine model of BA introduced by Riepenhoff-Talty et al [10] results in clinical and histologic features similar to that of the human disease and has subsequently become an important tool in BA research. In this model, neonatal infection of BALB/c (Harlan Labs, Indianapolis, Ind) mice with rhesus rotavirus (RRV), a double-stranded RNA virus, leads to an obstructive cholangiopathy and subsequent signs of disease including jaundice, bilirubinuria, and acholic stool. Our group has previously demonstrated colocalization of RRV in biliary epithelial cells (BECs) resulting in an 81% mortality rate [11]. Recent research has begun to bridge the pathophysiologic gap between a possible viral etiology and the subsequent T-cell–mediated destruction of BECs. Harada and colleagues [12] demonstrated in vitro and in vivo that human BECs mount an antiviral response and initiate apoptotic pathways in response to a synthetic ds-RNA analog.

Rotavirus is a ubiquitous human pathogen and the leading cause of severe diarrheal illness in children worldwide. In 2004, the World Health Organization estimated that rotaviral gastroenteritis was responsible for approximately 527,000 deaths, mostly occurring in developing nations [13]. The Centers for Disease Control estimate that rotavirus is responsible for nearly 50,000 hospital admissions of children younger than 5 years in the United States per year [14]. As a result, significant efforts were made to develop a viable rotavirus vaccine. These efforts came to fruition with the Food and Drug Administration's approval of 2 live rotavirus vaccines for use in humans, Rotateq (Merck and Co Inc, Whitehouse Station, NJ) in 2006 and Rotarix (GlaxoSmith-Kline, Philadelphia, PA, USA) in 2008. Since then, phase III clinical trials have demonstrated these vaccines' ability to significantly decrease rotavirus gastroenteritis of any severity as well as severe cases of rotavirus gastroenteritis [15-18].

The aim of this study was to determine whether maternal postconception vaccination could prevent the murine model of BA and to determine the mechanism that mediates this protection. To do so, we monitored RRV-infected pups born to vaccinated dams for symptoms related to BA and established whether these clinical findings correlated with any histologic appearance of biliary tract damage. We also determined viral presence in pup biliary trees and collected maternal and pup serum for quantitative rotavirus IgG and IgA levels as well as anti–RRV-neutralizing antibody (NA) titers. We also performed postconception vaccination studies on a B-cell–deficient strain of mice to determine the role of rotavirus antibody.

1. Materials and methods

1.1. Rotavirus strains

Rotavirus strains have been characterized according to differences in structural proteins and classified into 7 major groups (A-G). For these studies, we used 2 strains of group A rotavirus—the simian strain RRV, obtained from Dr Harry Greenberg (Stanford University, Palo Alto, Calif), and the human strain Wa (kindly provided by R Wyatt, National Institutes of Health, Bethesda, Md). We included Wa because, in previous studies, it has been shown that Wa, a human group A rotavirus strain, does not induce the murine model of BA [11]. Of the group A rotaviruses, Wa G1P [8] and the simian strain RRV G3P [3] demonstrate the least homology of proteins VP4 and VP7 [19]; thus, we hypothesized that Wa injection would not elicit NAs against RRV. The 2 strains of rotavirus were maintained in the monkey kidney epithelial MA104 cell line. The concentration of each strain was determined by focus forming viral titration assays. Rhesus rotavirus and Wa obtained from cell culture lysates were used for live virus immunization.

1.2. Rotavirus vaccine

Rotateq is a live pentavalent rotavirus vaccine (PRV) containing 5 human-bovine rotavirus reassortants approved for oral use in human infants for the prevention of rotavirus gastroenteritis. The murine maternal dose was estimated by first calculating the average cumulative dose received by a human infant normalized by weight as the vaccine is intended to be given as a series of doses. This weight-based dose was then adjusted to average adult female mouse weight and yielded a dose of 60 μL. Prenatal postconception immunization was then carried out as described below. A lower dose (30 μL) of vaccine was also injected to assess dose response.

1.3. Recombinant rotavirus protein

VP6 is a protein that comprises the intermediate layer of the rotavirus particle and possesses a highly conserved genomic sequence across group A rotavirus species [20]. Previous studies have demonstrated that mucosal administration of recombinant VP6 protein with an adjuvant to adult mice elicited a protective immune response against subsequent oral rotavirus challenge [21]. Given its antigenic properties and cross-strain homology, a recombinant form of this protein has been developed as a nonliving rotavirus vaccine candidate [21,22]. Synthesis of the plasmid containing the VP6 of Epizootic Diarrhea of Infant Mice (EDIM), a murine strain of rotavirus, expression of VP6 as a chimera with maltose binding protein (VP6::MBP), and purification of the protein have been previously described [23]. A total dose of 9 μg of protein was administered to pregnant dams.