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Prevention of Cholestasis in the Murine Rotavirus-Induced Biliary Atresia Model Using Passive Immunization and Nonreplicating Virus-Like Particles

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Abstract

Biliary atresia is a neonatal cholangiopathy of unknown etiology that results in obliteration of bile ducts and is the primary indication for liver transplant in children. A murine model of BA, which involves infecting newborn mice with rhesus rotavirus (RRV) and leads to development of an obstructive cholangiopathy, has provided a model to assess measures to prevent and treat BA. We used this mouse model of RRV-induced BA to determine if passive immunization of pups using maternal immunization [injection of dams with non-replicating rotavirus (RV) virus-like particles (VLPs) or live RRV] or injection of pups with RV immune serum would protect these RRV-infected neonates from developing BA (measured using cholestasis). Parenteral immunization of mouse dams with two formulations of VLPs (containing viral proteins 2/6 or 2/6/7) resulted in a significant increase in serum RV antibody, and pups born to these immunized dams were protected from developing cholestasis following neonatal infection with RRV. Serum RV-specific antibody with titers of 400-800 in dams significantly protected pups from developing cholestasis, and a significant trend of increasing protection with high titers was observed ($p > 0.0001$). Cholestatic pups had lower levels of RV serum antibody and higher serum ($p > 0.01$) and liver ($p > 0.05$) RV antigen compared to healthy pups. Passive transfer of low-titer (1600; $p > 0.05$) or high-titer (25,600; $p > 0.01$) RV immune serum to neonatal pups prior to RRV infection also protected them from developing cholestasis. Together, these findings indicate that passively acquired, neutralizing or non-neutralizing RV serum antibody attenuates viral replication and protects pups against disease in the RRV BA model. Early reduction of viral load by clearance with RV-specific antibody is likely a critical determinant of disease in this model.

Keywords

biliary atresia; rotavirus; virus-like particles; maternal immunization; immune serum

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Introduction

Biliary atresia (BA) is an idiopathic neonatal cholangiopathy that results in progressive fibro-inflammatory obliteration of intrahepatic and extrahepatic bile ducts that is the most common indication for liver transplant in children [1]. A mouse model of rotavirus (RV)-induced BA, in which rhesus rotavirus (RRV) infection of biliary epithelium triggers immune-mediated obliteration of bile ducts, has improved our understanding of the pathogenesis of this disease [2-6].

Intraperitoneal immunization of mouse dams in the RV model of BA using live virus, RV capsid protein VP6, or the currently licensed, live, attenuated RV vaccines has been shown to be effective in preventing BA in RRV-infected pups [4, 8, 9]. Maternal immunization (during pregnancy) is an effective and safe means of eliciting or increasing maternal antibody to infectious diseases that can have serious consequences for infants [10]. Rotavirus virus-like particles (VLPs) are candidate vaccines that consist of various combinations of the major viral structural proteins (e.g. VP2, VP6, VP7) that self-assemble into structures resembling native viral capsids [11]. They are safe and immunogenic when administered parenterally [12, 13]. In the studies described herein, we sought to determine if maternal immunization using RV VLPs would protect RRV-infected neonatal mice from developing signs of BA (cholestasis), if neutralizing antibody is needed, and if there is a threshold maternal anti-RV antibody titer required to protect pups from developing signs of BA. Finally, we tested the protective effect of administration of RV immune serum to pups.

Materials and Methods

Virus-like particle vaccines and viruses

2/6 and 2/6/7 virus-like particles (VLPs) were prepared in insect cells and purified as previously described [11]. The simian rhesus RV (RRV) (P5[3], G3) was kindly supplied by H. B. Greenberg (Stanford University Medical School, Palo Alto, CA) and was cultivated as described previously [14].

Animal Inoculations, Assessment, and Tissue Processing

For the maternal immunization experiments, RV-naïve BALB/c dams received 20 µg injections of either RV 2/6 or RV 2/6/7 VLPs plus 10 µg QS21 adjuvant (Wyeth, formerly in Madison, New Jersey) or 10 µg QS21 suspended in a 40 µl of 10 mM Tris, 150 mM NaCl, 10 mM CaCl₂ ("adjuvant alone" animals). All injections were into the hindlimb muscle. Following immunization, dams were paired with BALB/c males for mating. Between 24 and 48 hours following birth of pups, blood was taken from the tail veins of dams for determination of peripartum serum RV antibody titer, and newborn pups were inoculated intraperitoneally (ip) with 10⁷ pfu live RRV or with saline (controls) as described previously [14]. For immune serum experiments, serum was collected from RV-naïve mice ("non-immune") or from mouse dams that had previously raised RRV-infected pups ("high-titer"). An aliquot of the immune serum was diluted 1:10 ("low-titer"). Immune serum preparations were titrated using ELISA (see below) and 60 µl of each were administered i.p. to pups within 12-24 hours of birth. Twenty-four hours later, pups were inoculated with 10⁷ pfu RRV. Cholestasis, which occurs in BA as a result of bile duct obstruction, was assessed as described previously, and defined as presence of acholic stool and/or bilirubinuria at time of euthanasia. This method was chosen because these measurements correlate completely with subsequent abnormal bile duct morphology [14]. Serum and tissues were harvested at 11 dpi as described previously [14]. Dams and pups were coded with numbers/letters that did not reflect immunization status, and coded samples were harvested and analyzed further.

Detection of serum total RV antibody by ELISA

ELISAs were performed as described previously [14]. A dilution was considered positive if the OD₄₅₀ value of the serum tested from infected animals in the virus well minus the control well was greater than the mean plus 3 standard deviations of the virus well minus control well for all RV-naïve mouse serum samples tested (N=21). Samples that did not have titers of at least 50 (the lowest dilution) were designated as having titers of 25.

Detection of RV antigen by ELISA

Serum and liver homogenate samples were evaluated for RV antigen by ELISA as described previously [14]. A cutoff value for detectable antigen was defined as greater than or equal to the mean of the ODs of the PBS control animal samples plus 3 standard deviations.

Immunoblot Analysis

Boiled lysate from RRV-infected MA104 cells was loaded onto a 4-20% gradient polyacrylamide gel (BioRad), transferred onto nitrocellulose membranes, and blocked as previously described [16]. Proteins were detected using the “high-titer” immune serum diluted 1:100 in 0.5% blotto.

Statistical analysis

Mann-Whitney U test was used to compare maternal pre-vaccination and post-vaccination titers as well as serum antibody titers of sick versus healthy pups. Chi square with Cochran-Armitage trend test was used to determine significant trends in percent cholestatic mice based on maternal antibody titers and based on maternal vaccination given. Z-test for comparison of two proportions was used to determine significant differences in proportion of cholestatic mice between two groups. Z-test for comparison of two proportions was also used to compare rates of cholestasis among litters born to dams with different RV antibody titers and to determine a protective antibody threshold. Wilcoxon test was used to determine differences in antibody titers between healthy and diseased pups. Student's t-test was used to compare serum and liver RV antigen levels. All statistical tests were two-tailed/two-sided.

Results

Immunization of mouse dams with RV virus-like particles induces RV-specific serum antibody titers

The immunogenicity of two types of RV VLPs were tested: 2/6/7 VLPs, which elicit neutralizing antibodies to viral protein VP7 and non-neutralizing antibodies to VP2 and VP6, and 2/6 VLPs, which elicit non-neutralizing antibodies to VP2 and VP6 [12, 17, 18]. 2/6 VLPs were tested only in RV-naïve dams, and 2/6/7 VLPs were tested in both naïve dams and in RRV-exposed dams (dams that had previously raised RRV-infected pups). Serum antibody titers were induced in all naïve dams given 2/6 VLPs (Fig. 1, middle panel). GMT also increased significantly in both naïve and RRV-exposed dams given 2/6/7 VLPs (from 69 to 5,298). A seroresponse (at least four-fold rise in antibody titer) occurred in all naïve dams immunized with either 2/6 ($p>0.05$) or with 2/6/7 VLPs ($p>0.001$). Two of the three (67%) RRV-exposed dams immunized with 2/6/7 VLPs responded with 6-fold or greater increases in titer. The single RRV-exposed dam that was a nonresponder to 2/6/7 VLPs had the highest pre-vaccination antibody titer (6400), and no rise in antibody titer was detected in any of the control animals given adjuvant alone (Fig. 1, left panel). These data show that RV 2/6 VLPs and 2/6/7 VLPs are immunogenic, that they significantly increased RV antibody titers in naïve dams, and that 2/6/7 VLPs further increased the titers of previously RRV-exposed mice.

Pups born to dams exposed to live RRV or immunized with 2/6/7 or with 2/6 VLPs pre-pregnancy are protected against RRV-induced cholestasis

We previously showed that neonatal mice inoculated with RRV have replicating virus in biliary epithelium and RV antigen in serum at 10-11 dpi, and that they develop BA [14]. Here, to determine whether specific levels of maternal anti-RV antibody protected pups from developing cholestasis, we infected pups born to 21 RV-naïve dams that were immunized with adjuvant and/or RV VLPs and pups born to 10 RRV-exposed dams (of which 3 had also received 2/6/7 VLPs). Of 27 pups born to 9 RV-naïve dams that received adjuvant only, almost all pups (93%) developed cholestasis (Fig. 2a). Rates of cholestasis in each of the three other maternal treatment groups – 2/6 VLP vaccination, 2/6/7 VLP vaccination, and RV exposure – were significantly lower than in the adjuvant-only group ($p > 0.001$). There was a significant trend of increasing protection comparing the adjuvant (93% of pups developed cholestasis) to 2/6 VLP (33% cholestasis) to 2/6/7 VLP (17% cholestasis) treatment groups, with the 2/6/7 VLP group being most highly protected ($p > 0.001$). The finding that immunization with 2/6 VLPs protects RRV-infected offspring from developing cholestasis indicates that a neutralizing antibody response is not essential, although pups born to 2/6 VLP-immunized dams (33% cholestasis) had significantly higher rates of cholestasis than those born to RRV-exposed dams (9% cholestasis) ($p > 0.05$).

Low levels of maternal serum RV-specific antibody are protective against cholestasis in RRV-infected pups

To further characterize the immune status of dams and its effect on prevention of cholestasis in offspring, percent cholestatic pups was evaluated based on level of maternal peripartum RV antibody. Pups born to dams with undetectable peripartum serum RV antibody titers (i.e. assigned titers of 25) had significantly higher rates of cholestasis than did pups born to dams with peripartum RV titers of 100 or higher ($p > 0.01$) (Fig. 2b). Increasing maternal antibody titer levels were significantly associated with lower rates of cholestasis in pups ($p > 0.0001$). In a separate analysis of the subgroup of pups of naïve dams immunized with 2/6/7 VLPs or 2/6 VLPs compared to adjuvant only, pups born to naïve, non-immune dams had significantly higher rates of cholestasis (93%) than pups born to dams with peripartum anti-RV titers of 400 or higher (24%) ($p > 0.01$) (Fig. 3). Furthermore, there was a significantly lower proportion of cholestatic pups born to dams with titers of 400-800 (57%) than those born to dams with undetectable (i.e. titer=25) titers (93%), indicating a protective maternal antibody titer threshold in the range of 400-800. These results suggest that RV-infected pups born to dams with even relatively low RV-specific antibody titers are protected from cholestasis.

Cholestatic pups have significantly lower serum RV antibody titers at 11 dpi than healthy pups

Sera were obtained from a subset of pups at 11 dpi for determination of RV-specific antibody titers. The 11 dpi timepoint allows for assessment of all pups that develop cholestasis as well as detection of RV in the liver and blood [7, 14, 15, and unpublished data]. The ranges of serum antibody titers for healthy and cholestatic pups overlapped (Fig. 4), but cholestatic pups had significantly lower mean antibody titers (GMT of 1,433) compared with healthy pups (GMT of 7,313) ($p > 0.001$). Only 2 healthy pups were born to naïve, unvaccinated dams. These data illustrate that the protection against cholestasis observed in the RRV-infected pups born to dams with anti-RV antibodies is mediated by transmission of maternal antibody to pups, as reflected by their higher anti-RV antibody GMTs. Furthermore, several pups born to non-immune dams had antibody titers of up to 6400 and two pups born to non-immune dams had titers of 800 and were protected from

developing cholestasis, suggesting that the active pup humoral response may supplement protection provided from maternal antibody.

Cholestatic pups have significantly higher levels of serum and liver RV antigen than healthy pups at 11 dpi

We hypothesized that pups with RV-specific antibody would clear RV more effectively and that a lower viral load might result in lower rates of cholestasis in these animals. RV antigen in the serum, or antigenemia, is detected in humans and mice infected with RV and in bile duct epithelium in the RRV mouse model of BA [14, 19, 20]. RV antigen levels were thus determined in serum and in liver homogenates obtained at 11 dpi. Cholestatic pups had significantly higher levels of RV antigen in both serum (Fig 5a, $p>0.01$) and in liver homogenates (Fig 5b, $p>0.05$) than did healthy pups. These data indicate that, overall, serum and liver of cholestatic pups contained more RV antigen than healthy pups at 11 dpi, although many cholestatic pups had low or undetectable RV at this timepoint. This is not surprising, as RV appears to be cleared from the blood and liver in this model by approximately 2 weeks post-inoculation [7, 14, 15]. No healthy pups had high levels of RV antigen in serum or liver at 11 dpi.

Passive administration of RV immune serum to pups prior to infection can prevent RRV-induced cholestasis

In a simian model of RV infection, intravenous infusion of RV immune serum attenuated fecal shedding of RV after oral challenge of animals aged 3-6 months [21]. To determine whether passive transfer of RV immune serum can protect pups from developing cholestasis, pups were administered RV immune serum (high-titer or low-titer) or non-immune serum by i.p. injection at 12-24 hours of life followed by injection with RV 24 hours later. To quantitate serum RV antibody in pups inoculated with each type of serum at the time of RRV inoculation, an additional set of pups was injected with immune serum at 12-24 hours of life and then euthanized for serum collection 24 hours later. The serum anti-RV antibody titers (GMTs) for these mice were: 898 for pups given high-titer serum (N=6), 400 for pups given low-titer serum (N=5), and 59 for pups given non-immune serum (N=8). We also determined the immunoreactivity to RV proteins of the high-titer immune serum by performing a Western blot using RRV-infected cell lysate (Fig. 6a). The blot, which shows bands representing RV proteins recognized by the immune serum, revealed that antibody to both structural (VP2, VP4, VP6, VP7) and nonstructural (NSP2 and NSP4) proteins was present in the immune serum. Pups inoculated with non-immune serum had significantly higher rates of cholestasis (74%) than pups inoculated with low-titer serum (27%; $p>0.01$) or high-titer serum (17%; $p>0.01$) and there was a significant trend of increasing protection with increasing anti-RV titer of immune serum ($p>0.01$) (Fig. 6b). Together, these data indicate that passive transfer of low- or high-titer RV immune serum to newborn pups circulates in the blood and can protect pups from RRV-induced BA.

Discussion

In the present studies, we have demonstrated the following novel observations in the RRV mouse model of BA: (1) RV VLPs are efficacious when used as a parenteral maternal vaccine to prevent cholestasis in RRV-infected pups; (2) neutralizing antibodies (elicited using live RV or 2/6/7 VLPs) and non-neutralizing antibodies (elicited using 2/6 VLPs) are able to protect against cholestasis, although there was a significant trend toward increasing efficacy with 2/6/7 VLPs compared with 2/6 VLPs (and compared with adjuvant alone); (3) low levels of RV-specific maternal antibody are protective, with a protective maternal antibody titer threshold range of 400-800; (4) RV antigen is reduced in serum and in the liver in protected pups; (5) cholestatic pups have lower serum RV antibody and higher

serum RV antigen than healthy pups, supporting that antibody-mediated clearance of RV antigen is an important determinant of disease prevention in this model of extraintestinal RV disease; and (6) injection of pups with RV immune serum prevents cholestasis in the RRV BA model.

We are the first to show that VLP vaccines prevent cholestasis in the RRV mouse BA model. Other investigators have studied maternal immunization in this model using two currently licensed live RV vaccines as well as live RRV, human RV strain Wa, and a recombinant RV capsid protein VP6 [4, 8, 9]. We also are the first to immunize dams in this model by an intramuscular route rather than the intraperitoneal or oral routes used previously [4, 8, 9]. The route of immunization is important because live virus was detected in the placentas and fetal tissues of dams injected intraperitoneally with live virus [4]. Thus, it is unlikely a live virus vaccine (such as the currently licensed RV vaccines: Rotarix® (GlaxoSmithKline), an attenuated human RV strain; or RotaTeq® (Merck & Co., Inc.), which consists of five recombinant rotaviruses on a bovine background) or any vaccine given by an intraperitoneal injection route would ever be used as a maternal vaccine, but parenteral immunization with a nonreplicating immunogen such as a VLP vaccine could be safely used in pregnant women as is done with inactivated influenza vaccine.

Our finding that pups of dams lacking detectable anti-RV antibodies have high rates of disease in the RRV mouse BA model is consistent with a previous report demonstrating that pups of RV-immunized B cell-deficient dams, which cannot produce antibody, are not protected against disease following RRV infection [8]. In our studies, however, using 2/6 VLPs as a vaccine, we also showed that *neutralizing* antibody is not required for protection against disease in this model. Despite lack of a neutralizing antibody response in our 2/6 VLP immunized dams, RRV-infected pups born to these dams had detectable RV-specific antibody and were protected against disease. Rotavirus non-neutralizing antibodies have been reported to correlate with protection, and some data provide possible explanations. For example, in children, levels of neutralizing antibodies do not always correlate with protection from diarrheal disease [22, 23]. 2/6 VLPs, while lacking capsid proteins that elicit a classically neutralizing antibody response, and a VP6 immunogen (tested previously in this model), can protect against RV infection and BA [8, 18]. The mechanism of protection with antibody to VP6 may be related to its ability to inhibit transcription of mRNAs from the RV capsid [24,25] and this occurs intracellularly at an early stage of infection [26].

In addition to studying the effects of different maternal vaccines on protection against disease in pups, we found that low maternal antibody titers are sufficient to significantly protect pups, with a protective maternal antibody threshold of between 400 and 800. We also measured RV-specific serum antibody levels in our pups and examined the relationship between pup antibody level and disease development, which has not been done previously. Importantly, almost all of the pups born to non-immune dams developed cholestasis, and a majority of pups born to immunized dams remained healthy, regardless of pup antibody titers. This suggests that maternal antibody, which is the sole source of RV-specific antibody in early stages of infection, is a more important determinant of protection than the pup humoral response.

Previous studies have shown that viral infection of cholangiocytes is a critical initiating event in pathogenesis in the BA mouse model, and it is thus not surprising that early presence of RV-specific antibody can prevent disease in the model. Following cholangiocyte infection, programmed T cells and NK cells from affected mice inflict biliary epithelial damage [6, 27, 28]. Infection of cholangiocytes is critical in initiating these cytotoxic events. Using reassortant RV strains, Wang et al. showed that the RV VP4 gene, which mediates RV attachment to cells, is the critical viral determinant of BA in the mouse model [29]. This

indicates that RV binding and infectivity is a critical early component of pathogenesis in this model, and supports the hypothesis that antibody-mediated clearance of RV early in the disease process can attenuate disease, as observed in our experiments.

We also measured RV antigen in the serum and livers of infected pups for the first time in maternal immunization experiments in this model, and determined that protected pups had lower levels of virus. Other authors have measured RV in the extrahepatic bile ducts or in liver homogenates of RRV-infected pups born to immunized dams [8, 9]. Bondoc et al. reported lower levels of RV in the bile ducts of pups born to immunized dams compared with non-immune dams on post-infection days 2 and 7 [8]. In contrast, Turowski et al., found comparable levels of RV in livers of pups born to immunized dams and non-immune control dams [9]. Our finding that healthy pups had lower RV antigen levels than cholestatic pups is consistent with the findings of Bondoc et al. [8] and, similarly, suggest that antibody-mediated clearance of RV in healthy RRV-infected pups likely plays a significant role in protection from disease in this model.

To further examine the protective role of passively-acquired antibody in the RRV BA model, we transferred RV immune serum by intraperitoneal injection into pups prior to infection, and found that this was also effective in preventing cholestasis. Passive transfer of IgG into naïve pigtailed macaques has previously been shown to suppress or delay viral infection [21]. In these experiments, the investigators infused RV-specific IgG intravenously and then challenged with oral RV, and found that fecal RV antigen shedding was attenuated compared with animals that received non-immune serum prior to challenge. Although we did not test antigen in pups injected with immune serum, our finding that pups born to dams with high antibody levels have less RV antigen in serum and liver are consistent with the results in the macaque experiments and, together, show that RV-specific antibody is a critical factor in clearance of RV in these models. One possible caveat to our immune serum experiment findings is that we injected both the immune serum and then RRV (24 hrs later) into the peritoneal cavity, and it is possible that some immune serum remained in the peritoneal cavity at the time of RRV injection, neutralizing it prior to entry into the circulation. However, we determined that significant amounts of antibody were present in the serum 24h after injection of the immune serum (results section). If a small amount of antibody remained in the peritoneum at the time of RRV injection, it was unlikely to be of sufficient quantity to significantly neutralize RRV prior to its entry into the circulation. We also did not directly rule out the possibility that serum constituents in addition to RV-specific antibody, such as complement, or non-RV-specific antibody, might have played a role in the protective effect of the immune serum. However, this seems unlikely as such constituents would also have been present in the non-immune serum.

Previous studies in the RRV mouse BA model show that cellular immune responses are critical for disease pathogenesis [6, 28]. A role for a humoral immune response has also been proposed in the RRV mouse BA model based on antibody deposits found in and around bile duct epithelium, and antibodies cross-reactive with both RV and with the cholangiocyte protein alpha-enolase have been reported, indicating that molecular mimicry might play a role in the humoral response in this model [27,30]. In our experiments, passive administration of RV-specific antibody played a protective rather than pathogenic role. This may be because, in our experiments, the antibodies were present prior to injection of RV, and likely impaired viral replication at a very early stage, decreasing cholangiocyte infection and slowing the initial cascade of pathogenic events. One critical aspect of our immune serum experiment is that we injected the immune serum prior to RRV infection, which allowed very early viral clearance to occur, and prevented development of BA. In humans, however, BA is not diagnosed until disease is advanced; i.e. at a point where the cellular immune response has already inflicted significant damage to the bile ducts, which are

usually completely blocked by the time the disease is diagnosed. Thus, in human BA, treatment with immune serum at diagnosis may be too late to have a beneficial effect, although maternal immunization could be a realistic preventive measure.

Conclusions

Overall, our current studies and others reported previously indicate that RV-specific antibody is an important determinant of disease in the RRV mouse BA model. We have found that even low levels of antibody are protective, although it is not clear what functional properties of these antibodies are providing protection. The observations that immunization with the single viral capsid protein VP6, or with 2/6-VLPs, which induce non-neutralizing antibody and are sufficient for protection, suggest that perhaps viral clearance alone plays a major role in protection in this model [8, the current study].

Our finding that passive transfer of RV immune serum is protective not only validates previous reports and reinforces the hypothesis that antibody is critical but, importantly, will allow future experiments to test antibodies to specific RV proteins to be performed to determine which antibodies and, based on their functional roles, which components of the RV cycle are necessary for development of BA.

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Highlights

- Passive immunization was studied in the rotavirus mouse model of biliary atresia.
- Maternal immunization with virus-like particles protects pups against disease.
- Administration of rotavirus immune serum to pups also protects against disease.
- Low antibody levels and non-neutralizing antibody are sufficient for protection.
- Antibody-mediated viral clearance is a key determinant of disease in this model.

Figure 1. Immunization with RV virus-like particles (VLPs) significantly enhances maternal RV-specific serum antibody titers

Serum RV-specific antibody titers were determined on RV-naïve (solid lines) or RRV-exposed (dotted lines) adult female mice at baseline (“Pre”) and following immunization (“Post”) with adjuvant alone or in combination with 2/6 VLPs or 2/6/7 VLPs. Geometric mean titers are shown below each group.

Figure 2. Maternal immunization with RV 2/6 or 2/6/7 VLPs or with live RRV prevents development of cholestasis in RRV-inoculated pups

Percent cholestatic pups are shown based on (a) type of maternal vaccine and (b) maternal serum antibody titer. Dams in the 2/6 VLP and 2/6/7 VLP groups were RV-naïve prior to vaccination; three of the dams in the RV group also received 2/6/7 VLP vaccine (a). There was a significant trend of increased protective effect from adjuvant only to 2/6 VLPs to 2/6/7 VLPs ($p > 0.001$). There was also a significant trend of increased protective effect (% cholestasis) with increasing maternal antibody titer ($p > 0.0001$) (b). Number of cholestatic pups/total pups is shown inside each bar.

Figure 3. Maternal anti-RV titers of at least 8,000-12,800 induced by 2/6/7 VLP vaccination protect RRV-infected pups from cholestasis

Each bar represents a single pup born to a RV-naïve dam. Dams injected with adjuvant all had peripartum titers of 25, and their pups are represented in the shaded area. Dams injected with adjuvant plus 2/6 VLPs or 2/6/7 VLPs all had peripartum titers of 400 or higher, and their pups are represented in the unshaded area. Only 15 of 62 pups (24%) born to dams immunized with 2/6 or 2/6/7 VLPs (in unshaded area) developed cholestasis, whereas 25 of 27 pups (93%) born to dams injected with only adjuvant (shaded area) developed cholestasis ($p > 0.01$).

Figure 4. Healthy pups have significantly higher serum rotavirus-specific antibody titers at 11 dpi than do cholestatic pups following neonatal RV infection
 Healthy and cholestatic pups based on pup serum anti-RV antibody titers at 11 dpi. Each marker represents titer of an individual pup. Unfilled markers represent pups born to dams with peripartum titers of 25 (i.e. undetectable).

Figure 5. Healthy pups have significantly lower serum RV antigen levels at 11 dpi than do cholestatic pups

Serum (a) and liver (b) RV antigen are shown. Each diamond represents one pup. Unfilled diamonds represent pups born to dams with serum peripartum anti-RV titers of 25. Lower limit of antigen detection of 0.1 is designated by a dotted line.

Figure 6. Serum from dams of RRV-infected pups contains antibodies to RV capsid and non-capsid proteins, and prevents RRV-infected pups from developing cholestasis when injected intraperitoneally

Western blot showing bands representing RV proteins recognized by the RV immune serum used to prevent cholestasis in mice given RRV after birth (a). “MW”=molecular weight marker. “RV”=cell lysate from RRV-infected MA104 cells. The immune serum was derived from blood from dams that had previously raised RRV-infected pups. Percent cholestasis in litters of pups injected with non-immune serum (anti-RV ELISA titer 25), low-titer (high-titer serum diluted 1:10, titer confirmed 3200) immune serum, or high-titer (51,200) immune serum one day before RRV inoculation is graphically represented (b).