Mechanisms of Protection Against Rotavirus Infection and Disease

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Abstract: Rotavirus is a double-stranded RNA virus composed of 3 protein layers. These layers contain structural proteins (eg, VP4, VP6, and VP7) that are involved in the induction of immunity. Despite extensive research in animal models and humans, the mechanisms and effectors of protection against rotavirus after either natural infection or vaccination remain unclear. Complicating factors include the variety of immunologic responses produced after both natural infection and vaccination, and the fact that animal models do not fully mimic the human immunologic responses, even when inoculated with homologous rotaviruses. Nevertheless, it appears that neutralizing antibodies have a role in protection against rotavirus infection and disease, but that other effectors, such as non-neutralizing antibodies and T cells, have important effector properties as well. These effectors likely have overlapping functions, thus providing enhanced protection. The results of further research to elucidate the immunologic mechanism of protection will provide insight into improving the efficacy of current vaccines.

Key Words: Reoviridae, rotavirus, gastroenteritis, rotavirus vaccine, antibody, protection

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DISCOVERY OF ANIMAL AND HUMAN ROTAVIRUSES

Rotavirus was first described as a disease in mice in 1947 based on large outbreaks in laboratory animals.1,2 In 1963, rotavirus was discovered in monkeys,3 and in the same year, the virus was first visualized in the intestinal epithelium of infected mice using electron microscopy.4 Human rotavirus, the primary cause of severe acute infantile gastroenteritis, was finally discovered in 1973 by Bishop et al in duodenal mucosal specimens obtained from hospitalized children.5 Based on the distinctive wheel-like appearance of these viruses, they were subsequently given the name “rota” viruses from the Latin for wheel.6

ROTAVIRUS STRUCTURE

Rotaviruses are 70-nm double-stranded RNA viruses that belong to the family Reoviridae.7 The virus is composed of 3 protein layers: outer, middle, and inner (Fig. 1).7,8 Two structural proteins, VP4 and VP7, comprise the outer layer. These define the P and G serotypes, respectively.5–8 VP4 and VP7 play a critical role in the induction of immunity, and therefore, in the development of effective vaccines. These structural proteins define the serotype of the rotavirus via their abilities to elicit neutralizing antibody responses in infected humans or animals. VP4 and VP7 can produce both serotype-specific and cross-reactive serotype responses.6–8 Neutralizing antibodies against VP4 and VP7 clearly play some role in the induction of immunity after natural infection and oral immunization with live rotaviruses, but they are not the only effectors of protection.8 The middle capsid layer is comprised of the structural protein VP6, which bears the group-specific antigenic determinants and appears to be the most immunogenic protein.6,8 The development of serum immunoglobulin A (IgA) or immunoglobulin G (IgG) antibodies against VP6 are indicative of protection after either natural infection or vaccination, although whether these antibodies produce protection or are merely markers of protection is not well understood.6,8

The inner layer surrounds the viral genome composed of 11 segments of double-stranded RNA.7 Six gene segments encode 6 structural proteins (VP1–VP4, VP6, and VP7), and 5 gene segments encode 6 nonstructural proteins (NSP1–NSP6).7,8 NSP4 is of clinical significance because it has been identified as an entero-toxin capable of inducing diarrhea.7

WHAT IS THE MECHANISM OF PROTECTION AGAINST ROTAVIRUS?

Despite extensive research, the mechanisms of protection against rotavirus via either natural infection or vaccination are not clearly defined.5,8 Two main lines of argument have been proposed. The first suggests that the protection is due to antibodies that recognize serotype-specific neutralization epitopes within outer capsid VP4 or VP7 proteins of the rotavirus particle.8,10 The second proposes that protection is due to effectors other than neutralizing antibody following natural infection.8,10 These competing theories have been the basis for differing approaches to the development of vaccines.

HOW DO WE IDENTIFY MECHANISMS OF PROTECTION?

One approach to studying mechanisms of rotavirus protection is through the use of animal models. Animal models have been instrumental in understanding immunity to rotavirus because they avoid many of the limitations associated with human studies. Studies in pigs and mice have been the primary models for studying rotavirus immunity, although neither fully mimics the human situation.

Overall, animal studies suggest that both cell-mediated and humoral factors are important in the resolution of ongoing infection and protection against subsequent infections.8 Gene knock-out studies have been used to evaluate the roles of antibodies and T cells in the development of immunity against rotavirus in mice. In general, these studies indicate that neutralizing antibodies probably have an important role in protection after live rotavirus infection, but that other effectors such as non-neutralizing antibodies and possibly T cells (CD8 and/or CD4) also seem to have important effector functions.8,11,12

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Disclosure: Dr. Ward is an inventor of the vaccine that has led to Rotarix and receives royalties from GlaxoSmithKline as a result. His laboratory has been the central laboratory for most studies leading to the development of Rotarix, RotaTeq, and RotaShield. Address for correspondence: Richard Ward, PhD, Cincinnati Children’s Hospital Medical Center; University of Cincinnati, 3333 Burnet Avenue, Cincinnati, OH 45229. E-mail: dick.ward@cchmc.org.

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HOW DO WE EVALUATE PROTECTION AGAINST ROTAVIRUS IN HUMANS?

Evaluating protection against rotavirus in humans involves prospective monitoring of subjects for rotavirus infections paired with periodic sampling of blood or feces. Samples are analyzed for the studied marker of rotavirus infection (eg, serum or fecal IgA), and data are then analyzed to determine if levels of the marker are correlated with protection. Several studies conducted in developed, less-developed, and developing countries have found correlations between serum rotavirus IgG and IgA after natural infection.13–15 Similar correlations have been found with levels of fecal rotavirus IgA.16 However, the association between rotavirus antibody levels and protection is not complete in these studies, suggesting that factors other than antibodies are important for providing immunity.

WHAT IS THE ROLE OF NEUTRALIZING ANTIBODIES IN NATURAL INFECTION?

The properties of rotavirus antibodies after natural infection, particularly their ability to neutralize rotavirus, remain unclear. Several reports suggest that protection after natural infection is related to the serotype of the infecting strain, whereas others suggest that protection against subsequent rotavirus infection is independent of the serotype of rotavirus that caused the first infection. For example, an early small study in Japan found that titers of serotype-specific neutralizing antibody of ≥1:128 were correlated with protection after natural infection.17 However, a large study in Bangladesh found that although protection after natural infection was associated with the presence of serum rotavirus IgG,15 heterotypic antibodies were the only antibodies that were independently associated with protection against subsequent infection.18 It must be noted that these studies assessed only the relationship between antibodies and protection with no investigation of other potential mechanisms (eg, T cell responses). Thus, it is possible that the correlation between antibodies and protection is merely a marker of protection, with the actual mechanism related to some other immunologic event.

DO POSTVACCINATION ROTAVIRUS ANTIBODIES PREDICT PROTECTION?

The correlation between postvaccination rotavirus antibody titers and protection against rotavirus infection is less evident than that seen with natural rotavirus infection. For example, in the initial large trial with the tetravalent vaccine RotaShield, only 18% to 22% of those vaccinated developed serum neutralizing responses to rotavirus serotypes G1 through G4. This was despite the development of detectable IgA responses in 80% to 90% of vaccinated individuals and efficacy greater than 70% against severe disease.19 Furthermore, there was no correlation between protection against specific serotypes and serotype-specific neutralizing antibodies.19

Similar results were observed in the subsequent pivotal US trial with RotaShield, with less than one-third of patients seroconverting to any of the G1–G4 rotavirus serotypes.20 Although there was an association between antibody titers and protection against rotavirus infection, the best correlate of protection was with heterotypic serum neutralizing antibodies. In addition, serotype-specific responses were associated with no greater protective effect than were responses that were not serotype specific. Thus, no specific antibody response could be consistently correlated with protection.20

There was also no clear correlation between protection and serotype-specific neutralizing antibody levels with the pentavalent reassortment RotaTeq vaccine. Neutralizing antibody responses were generally higher after immunization with RotaTeq vaccine than with RotaShield, with up to 86% reporting seroconversion to G1 (ie, ≥3-fold increase in titer), and more than 90% of patients having a ≥3-fold increase in serum antirotavirus IgA.21 However, there was no clear correlation between immune responses and protection against infection.21

The best correlation between rotavirus antibody responses after vaccination and protection against infection has been with the single-strain Rotarix vaccine. In 3 placebo-controlled trials, 61% to 91% of vaccinated infants developed rotavirus-specific IgA antibodies after 2 doses.22 Subjects that did not develop serum
rotavirus IgA responses after 2 doses of vaccine were significantly more likely to develop rotavirus infection compared with individuals who did not develop responses. Nevertheless, the proportion of individuals who were protected against severe rotavirus infection by Rotarix exceeded the proportion who achieved measurable serum rotavirus IgA responses. This suggests that serum rotavirus IgA levels only partially correlated with the degree of protection stimulated by immunization with Rotarix. Further supporting the presence of other immune components beyond neutralizing antibody in the mechanism of protection with Rotarix is the observation that Rotarix can elicit a high level of protection against fully heterotypic strains of rotavirus.

SUMMARY

Despite substantial research in both animal models and human vaccinees, the effectors of protection after either natural infection or vaccination are not fully known. In particular, the role of antibodies remains in question. In general, total rotavirus antibody titers in serum and stool specimens of naturally infected subjects have correlated with protection. In contrast, rotavirus antibody levels correlate with protection to a lesser extent after live rotavirus vaccination and there are differences in immune responses between different vaccines. Other effectors, such as non-neutralizing antibodies and possibly T cells, may play a role in protection after live rotavirus infection. Overall, it appears that immune effectors have overlapping functions and the protection against rotavirus is likely enhanced by this redundancy. Further studies are required to define the mechanisms of action of immunity against rotavirus infection. Although a complete understanding of the underlying mechanisms is not required to develop an effective vaccine, such knowledge would enhance the ability to make rational improvements in current vaccines.

REFERENCES