Host Factors Associated with Protection against Rotavirus Disease: The Skies Are Clearing

Paul A. Offit

Over the past several years, a number of studies have clarified aspects of rotavirus immunology and vaccinology previously considered controversial. In this review, studies that address the following questions will be summarized: Which host factors are responsible for recovery from acute rotavirus infection? Are the host factors responsible for recovery from acute infection the same as those necessary for prevention of infection? What is the relative importance of the nature of the inoculum (e.g., homologous or heterologous host virus and live or inactivated virus), route of inoculation, or virus serotype in vaccine development? What is the immunologic basis by which infection with 1 viral serotype protects against challenge with another serotype (heterotypic protection)?

For many years, the host factors that determine recovery from acute rotavirus infection and protection against reinfection were not definitively known. However, over the past 3 years, a clearer picture has emerged. Summarized below are recent studies of important questions relating to rotavirus-specific immunity. Development of the best rotavirus vaccine will require a thorough understanding of these issues.

How Are Rotavirus-Specific Immune Responses Generated at the Intestinal Mucosal Surface?

The generation of virus-specific immune responses at the intestinal mucosal surface involves a complex interplay between antigen-presenting cells (APC), helper T (Th) cells, and effector cells (plasma cells and cytotoxic T lymphocytes [CTL]) [1]. Although much remains to be learned about generation of rotavirus-specific immune responses, the model outlined below is likely.

Rotavirus enters the small intestine after passage through the stomach and replicates in mature villous epithelial cells of the small intestine. Virus generated at the intestinal mucosal surface attaches to and enters specialized epithelial cells (M cells), which transport virus antigens to APC within Peyer’s patches (PP). APC, such as B cells, macrophages, and dendritic cells, process and present antigens to naive Th cells. Once activated, Th cells in PP initiate expansion of virus-specific B cells and CTL precursors (CTLp). Th cells in PP promote a switch from virus-specific IgM- to IgA-bearing B cells. Virus-specific B cells and CTLp leave PP, enter draining lymphatics, and finally enter the circulation via the thoracic duct. B cells and CTLp “traffic” back to the small intestinal lamina propria via homing receptors on their surface and vascular addressins on the surface of lamina propria capillary endothelial cells.

In the lamina propria, secretion of virus-specific IgA by plasma cells is induced by cytokines such as interleukin (IL)-4, -5, and -6. In addition, differentiation of virus-specific CTLp to effector CTL is promoted by cytokines, including IL-2 and interferon. Therefore, whereas PP are the major site for induction of virus-specific immune responses in the intestine, the lamina propria is the major site for maturation of virus-specific effector cells. Mature, virus-specific plasma cells in the lamina propria secrete dimeric IgA, which attaches to polymeric immunoglobulin receptors on the basolateral surface of villous epithelial cells. IgA enters the cytoplasm of intestinal epithelial cells, acquires a secretory piece, and is secreted into the intestinal lumen. Virus-specific CTL are also transported to the intestinal mucosal surface, where they reside among villous epithelial cells. Virus-specific CTLp and memory B cells remain within the lamina propria.

Which Host Factors Are Responsible for Recovery from Acute Rotavirus Infection?

Resolution of rotavirus disease probably occurs by both non-immunologic and immunologic mechanisms. A number of epidemiologic and experimental observations support the contention that nonimmunologic factors may alone be associated with resolution of primary infection. First, rotavirus infections usually resolve within the first week of the onset of symptoms: fever lasts ~3 days, vomiting 2–3 days, and diarrhea 5 days [2]. However, infants acutely infected with rotavirus may not develop virus-specific secretory IgA (sIgA) in either duodenal fluid or feces within the first week of infection [3]. Second, virus-specific IgA is not detected in feces, intestinal washes, or intestinal organ cultures in mice within the first
week of infection. (Detection of virus-specific IgA in superna-
tant fluids from intestinal organ cultures is considerably more
sensitive than detection of virus-specific IgA in intestinal
washes or feces [4].)

Third, mice infected with murine rotavirus may resolve viral
shedding even if deficient in their capacity to generate both
virus-specific IgA (B cell–deficient gene knockout mice) and
virus-specific CTL (CD8 cell–depleted mice) [5]. (It should
be noted, however, that the capability for studies in adult mice
to predict factors associated with protection against disease in
humans is limited in that adult mice shed virus in the absence
of symptoms.)

There are probably at least three nonimmunologic mech­
anisms by which the host resolves a primary rotavirus infection,
although none have been proven in either humans or experi­
mental animals. First, mature villous epithelial cells are re­
placed during infection by epithelial cells that are less mature
and, therefore, probably less permissive to viral growth; virus
does not appear to replicate in relatively immature epithelial
cells located in villous crypts [6]. In addition, increased peri­
estalsis of the small intestine probably hastens clearance of in­
fec tious virus and decreases the time during which infectious
virus may come in contact with sus ceptible villous epithelial
cells. Last, interferons produced by infected villous epithelial
cells or intraepithelial lymphocytes may ablate translation of
viral proteins.

In many children, immunologic factors are also clearly im­
portant for the resolution of acute infection. Children with
severe combined immunodeficiency, common variable immu­
nodeficiency, X-linked agammaglobulinemia, or acquired im­
munodeficiency secondary to immunosuppressive therapy or
human immunodeficiency virus infection develop chronic diar­
rhea secondary to rotavirus infection [7–11]. The importance
of virus-specific sIgA or virus-specific CTL (or both) in resolu­
tion of disease is supported by several findings in experimen­
tal animals. Mice deficient in the capacity to produce virus-specific
sIgA develop chronic viral shedding after infection with murine
rotavirus [12]. In addition, several observations support the
possible importance of rotavirus-specific CTL in resolution of
acute infection: Rotavirus-specific CTL appear at the intestinal
mucosal surface in mice within the first week of infection [13],
resolution of viral shedding in adult mice unable to generate
rotavirus-specific CTL (β2-microglobulin gene knockout mice)
is delayed by several days [12], and adoptive transfer of virus-
specific CTL in suckling mice is associated with amelioration of
acute disease [14].

Are the Host Factors Responsible for Recovery from
Acute Infection the Same as Those Necessary for
Prevention of Reinfection?

Natural rotavirus infection protects against disease induced
by subsequent infection [15, 16]; indeed, protection against
disease is clearly predicted by the quantity of virus-specific
sIgA present at the intestinal mucosal surface (as reflected in
the feces) [17, 18] and the presence of virus-specific IgA in
the serum [19]. These findings are consistent with several ob­
servations. Rotaviruses replicate in mature villous epithelial
cells at the intestinal mucosal surface [20]. Virus is never con­
sistently detected in the circulation or in sites distant to the
intestine. Therefore, protection against reinfection must be me­
diated by immune responses active at the intestinal mucosal
surface. Given the host’s commitment to produce sIgA at mu­
cosal surfaces (~5 g of IgA is produced by adult humans each
day) [21], virus-specific sIgA would be a logical choice as the
effector function most likely to correlate with protection against
rotavirus disease. In addition, virus-specific IgA at the intestinal
mucosal surface and in serum correlates with reduced viral
shedding caused by reinfection in experimental animals [22–
24].

Although, for the most part, natural infection protects against
disease caused by reinfection, some children develop symptoma­
tic reinfections with the same rotavirus serotype during the
following rotavirus season (usually 1 year later in temperate
climates) [25–36]. In addition, a small number of children
develop symptomatic infections twice within the same rotavirus
season (lasting ~4–5 months) [17]. These observations are
consistent with the fact that the presence of virus-specific sIgA
at the intestinal surface is usually short-lived. Indeed, rotavirus-
specific sIgA is often not detected in the feces 1 year after
infection [18].

Whereas the presence of rotavirus-specific IgA in feces and
serum correlates with protection against disease in studies of
natural infection, this is not clearly the case in studies of immu­
nization (with animal rotaviruses or reassortants between ani­
mal and human rotaviruses). For example, immunization of
infants with rhesus rotavirus (strain RRV) clearly induces vi­
rus-specific IgA in serum and feces [37]. However, induction
of IgA in serum after immunization does not predict protec­tion
against disease [38–40]. (Unfortunately, detailed studies of the
capacity of virus-specific sIgA in feces to predict protection
against disease have not been reported.) Differences in the
capacity of IgA in serum to predict protection against disease
following natural infection or immunization may relate to dif­
f erences between the host response to homologous and heterol­
ogous host virus infections, respectively. Alternatively, these
differences may relate to the manner in which studies were
done. In studies of natural infection, sera and feces were col­
clected at regular intervals throughout the rotavirus season,
whereas in studies of immunization, sera were collected usually
within 1 month of the final dose. Therefore, the length of time
between serum collection and onset of disease in studies of
natural infection did not usually exceed 1 month, whereas in
studies of immunization, the interval could have been as long
as 5 months. Virus-specific sIgA in serum may be short-lived
and, therefore, an inaccurate predictor of IgA levels 4–5
months later. Future studies determining correlates of protection following immunization should include biweekly collection of feces through the rotavirus season.

Although high levels of rotavirus-specific sIgA at the intestinal mucosal surface may completely protect against symptomatic reinfection [17, 18], rotavirus-specific memory B and T cells located with the lamina propria are more likely important in modification but not prevention of disease (i.e., protection against moderate to severe but not mild disease). This hypothesis is supported by the fact that incubation periods for rotavirus infections are short (1–4 days). If virus-specific sIgA is located at the intestinal mucosal surface at the time of infection, virus would be neutralized before attachment to villous epithelial cells, and the host would be protected against mild, moderate, or severe disease. On the other hand, activation and differentiation of memory B cells and CTLp to antibody-secreting plasma cells and virus-specific CTL, respectively, may only occur after 3–4 days (i.e., after infection of disease); effector cells generated from memory cells may, therefore, shorten the duration of illness.

The relative importance of effector cells and memory cells in protection against rotavirus disease is probably similar to that observed in other infections with short incubation periods. For example, protection against disease after infection with "systemic" viruses, such as measles, mumps, rubella, or varicella, is usually lifelong and complete. On the other hand, protection against disease after infection with "superficial mucosal" viruses, such as rotavirus, influenza virus, and respiratory syncytial virus, may be short-lived (i.e., <1 year) and incomplete (i.e., protection against relatively severe but not mild disease). Whereas incubation periods for systemic virus infections are ~8–14 days, those for superficial mucosal infections are 1–4 days. Long but not short incubation periods allow ample time for activation and differentiation of memory cells to effector cells before inception of disease.

What Is the Relative Importance of the Nature of the Inoculum in the Generation of Protective Immune Responses?

Oral inoculation of experimental animals with homologous or heterologous host rotavirus strains induces production of virus-specific sIgA at the intestinal mucosal surface [22–24]. However, the degree to which virus is adapted to growth in intestinal villous epithelial cells (i.e., homologous host virus > heterologous host virus > inactivated virus) determines the relative capacity to induce virus-specific sIgA [22–24, 41]. Relatively large quantities of heterologous host viruses are required, compared with homologous host strains, to induce protective levels of virus-specific sIgA at the mucosal surface. There are several possible explanations for this difference. First, the generation of large quantities of infectious virus by strains well-adapted to growth at the intestinal surface may simply allow for greater quantities of virus antigen to enter PP for processing and presentation to the immune system. Second, homologous host viruses may be better adapted than heterologous host strains to uptake and processing by APC. Third, replication of virus in villous epithelial cells may, in addition, be associated with antigen presentation by those cells.

Studies of homologous and heterologous host rotaviruses in animals have clearly predicted findings in human vaccine trials. For example, primate rotaviruses are better adapted to growth in the human intestine than are bovine rotavirus strains: Rhesus rotavirus strain RRV and RRV-human reassortant viruses are detected in the feces of virtually all inoculated infants after immunization, whereas bovine strains or bovine-human rotavirus reassortants are detected in ~20% of stools after inoculation [42]. To generate sufficient quantities of rotavirus antigen in PP, primate rotaviruses (at a dose of 10^5 pfu) must replicate in villous epithelial cells. On the other hand, because bovine rotaviruses are poorly adapted to growth at the intestinal surface, larger inoculum size of bovine rotavirus may allow for similar quantities of primate and bovine antigens to enter PP for presentation to the immune system.

Oral inoculation of inactivated rotavirus in humans or experimental animals can induce virus-specific humoral immune responses at the intestinal mucosal surface [44]; however, large quantities of virus are required. (The need for large quantities of inactivated virus may be obviated by microencapsulation [45]). These observations support the hypotheses that APC in PP can process and present either infectious or inactivated virus and that villous epithelial cells are probably not required as APC.

What Is the Importance of Route of Inoculation in Generation of Immune Responses Active at the Intestinal Mucosal Surface?

Oral inoculation is more efficient than intramuscular inoculation at inducing virus-specific sIgA responses at the intestinal mucosal surface [46]. However, intramuscular inoculation with live or inactivated rotavirus induces IgA-secreting plasma cells in the small intestinal lamina propria [46]. In addition, protection against rotavirus shedding in rabbits following intramuscular inoculation has been found to correlate with the presence of virus-specific IgG at the intestinal mucosal surface [47]. Intramuscular inoculation may be of value for vaccine strategies that include agents likely to be significantly altered by exposure to gastric acid (e.g., purified proteins, virus-like particles, or inactivated viruses). In addition, intramuscular immunization should be considered for use in developing countries.
where high titers of rotavirus-specific antibodies in milk may interfere with the immunogenicity of orally administered vaccines.

**What Is the Importance of Virus Serotype in Vaccine Development?**

Similar to influenza viruses, the serotype of rotaviruses is determined by two surface proteins: VP4, a protein cleaved by the protease trypsin (hence, P type), and VP7, a glycoprotein (hence, G type). Both proteins contain epitopes that are serotype specific and broadly cross-reactive (reviewed in [48]). At least 4 G types and 3 P types have been found to be associated with most rotavirus strains isolated from infants worldwide [43].

After a primary rotavirus infection, infants and young children develop neutralizing antibodies in serum directed against the G type of the infecting strain and strains with different G types [49–53]; however, the titer of neutralizing antibodies after primary infection is usually several-fold greater against the G type of the infecting strain (homotypic response) than against strains with different G types (heterotypic response) [49–53]. Similarly, children naturally infected with rotavirus are more likely to be protected against challenge by a strain with similar than with different G types [54, 55].

Studies of immunization of infants have not clearly defined the importance of G type in protection against disease. However, recently a pattern has emerged. Heterologous host (i.e., nonhuman) rotavirus strains have been found to protect against serotypically distinct human strains. Primate strain RRV (P[3], G3) and bovine strains WC3 (P[5], G6) and NCDV (P[5], G6) have been found to protect against challenge by P[8], G1 strains (reviewed in [43]). However, protection has been inconsistent, with an efficacy of 0%–100% against moderate to severe disease [43]. In an attempt to enhance the consistency of protective immune responses, heterologous host viruses have been engineered to include either G or P types from human rotavirus strains (G1, G2, or G4 with RRV; G1, G2, or G3, or P[8] with WC3). With the inclusion of human surface proteins, animal-human rotavirus reassortants have consistently protected against human disease in vaccine trials [39, 43, 56–58]. Therefore, current studies support the inclusion of human G types in vaccine trials of animal-human reassortant strains. Formal proof of the importance of serotype (or the inclusion of genes that encode human surface proteins VP4, VP7, or both) awaits large studies that include a comparison of animal strains, animal-human reassortant viruses, and, perhaps, attenuated human rotavirus in settings where natural challenge is caused by >1 rotavirus serotype.

**What Is the Immunologic Basis by Which Infection with 1 Viral Serotype Protects against Challenge with Another Serotype (Heterotypic Protection)?**

Children infected with a particular rotavirus serotype are more likely to be protected against challenge with the same serotype [54, 55]. However, in some children, natural infection or immunization with a certain serotype can clearly induce protection against reinfection with different serotypes [54]. For example, infants immunized with bovine strain WC3 (P[5], G6) were protected against challenge with P[8], G1 strains; protection occurred in the absence of P[8], G1-specific neutralizing antibodies in serum [59]. There are several possible explanations for this observation. First, virus-specific sIgA at the intestinal surface may be directed against cross-reactive neutralizing epitopes on P[8], G1. Second, virus-specific non-neutralizing antibodies (e.g., directed against inner capsid protein VP6) may ablate infectious virus production during passage through villous epithelial cells on the way to the intestinal mucosal surface [60]. Third, incubation periods may have been long enough to allow generation of virus-specific CTL from CTLp within the lamina propria; virus-specific CTL have been found to broadly cross-react with different rotavirus serotypes [61, 62].

**Future Directions**

The daunting challenge to rotavirus investigators may be to formulate a vaccine that induces virus-specific IgA at the intestinal mucosal surface, which is both longer-lived and higher-titered than that induced after natural infection. Toward this end, it would be of value to study the effect of timing and frequency of booster dosing, including doses administered in the second year of life. It would also be of value to study agents that may prolong availability of antigen within PP (e.g., viral or bacterial recombinants expressing individual rotavirus genes or microencapsulation of putative vaccines) and alternative routes of immunization, including perhaps sequential parienteral or oral immunization.

**References**


