PERSPECTIVE

Immune Responses to Rotavirus Infection and Vaccination and Associated Correlates of Protection

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Group A rotavirus (RV) strains are a major cause of acute gastroenteritis (AGE) in infants and young children worldwide [1]. RV disease accounts for more than one-third of all diarrhea-related hospitalizations and 500,000–600,000 deaths per year [2–4]; most deaths occur in sub-Saharan Africa and Asia [3, 4]. Direct medical and indirect annual costs associated with RV disease are estimated to be €400 million in Europe [5–7] and to exceed US$ 1 billion in the United States [8].

RV strains form a genus of the *Reoviridae* family and possess a genome of 11 segments of double-stranded (ds) RNA, encoding 6 structural viral proteins (VPs) and 6 nonstructural proteins (NSPs). The infectious particle (ie, virion) consists of 3 layers: the inner layer (core) contains the viral genome, the viral RNA-dependent RNA polymerase (RdRp, VP1), the capping enzyme (VP3), and the scaffolding protein (VP2); the core is surrounded by a middle layer (VP6), and the outer layer consists of VP7 and VP4 [9].

RV infects mature enterocytes in the small intestine. Viral replication leads to increased intracellular Ca^{2+} level (effected by NSP4), increased Cl^- secretion, and shut-off of host cell protein synthesis (effected by NSP3), resulting in acute osmotic and secretory diarrhea (described in [9]). Various RV genes have been implicated in the pathogenesis of AGE [10]. After RV infection, a viremic stage of, at present, unclear significance has been identified in humans and experimental animals [11–13].

The RV-encoded NSP1 blocks interferon (IFN) production by various pathways [14–17]. RV infection downregulates the IFN- and pro-inflammatory cytokine–associated pathways in calves [18].

RV strains have a high genomic and antigenic diversity and are classified into at least 7 different groups (A–G), distinguished by different VP6. Most human RV infections are caused by group A RV strains, which are further subdivided into at least 2 subgroups (I, II), 23 G types (determined by VP7, a *g*lycoprotein), and 31 P types (determined by VP4, a *p*rotease-sensitive protein) [9, 19–21].

RV strains with different G and P types cocirculate and change in geographical regions over time [22–25]. In temperate climate regions, most cocirculating RV strains are types G1–G4 and G9 (typically G1P1A[8], G2P1B[4], G3P1A[8], G4P1A[8], and G9P1A[8]), but other G types (G5, G8, G10, and G12), in combination with various P types, may be most prevalent in tropical areas [21, 23, 24].

Nonspecific (innate) and acquired virus-specific humoral and cellular immune responses are elicited by RV infection [26, 27] or RV vaccination [28–33]. Although currently licensed vaccines are highly efficacious in protecting children from severe RV AGE, the molecular mechanisms of protection are not fully understood. This article considers the immune responses to natural RV infection and RV vaccination in both experimental animals and humans as potential correlates of protection.

IMMUNE RESPONSES AFTER RV INFECTION IN ANIMALS

Mouse

Although mouse pups can be infected with RV and develop disease, adult mice are an infection-only model [34]. In adult mice, B cells producing IgG and IgA, either systemically or mucosally, are critical for clearing RV infection [34].

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B cells expressing the intestinal homing receptor α4β7 are important for protection [35–37]. Passive transfer of RV type-specific neutralizing (NT) antibodies clears RV infection in a dose-dependent manner [38, 39]. Vaccination of mice with reassortant RV strains carrying VP4 and VP7 from different parental virus strains elicits antibodies directed against both VP7 and VP4, conveying protection from disease by each parental virus strain [39]. Protective RV-specific antibodies do not necessarily neutralize in vitro. Polymeric VP6-specific IgAs confer protection, possibly by intracellular inhibition of viral transcription (ie, intracellular neutralization) [40–43]. Work with genetically J-chain deficient mice suggested that transport of RV-specific IgA through epithelial gut cells is involved in the development of protection [44]. Neutralization of RV by IgA has only been demonstrated for VP4-specific antibodies [45, 46]. Heterologous, llama-derived, single-chain antibody fragments directed against VP6 cross-neutralize in vitro and partially protect mice in vivo [47]. Local IgA production may not be required for effective protection in mice; protective antibodies are generated by B cells expressing the gut homing receptor α4β7, even in mice unable to produce IgA [48, 49]. In severely immunodeficient mice, clearance of RV infection can be achieved by adoptive transfer of immune $CD8^+$ T lymphocytes [50, 51], based on the presence of cross-reactive T cell epitopes on VP7 [52], VP4, and VP6 [53]. Studies with mice carrying different immunological knockout lesions have demonstrated that B cell–dependent humoral immunity appears to be the main mechanism of protection from RV infection [34, 54–56]. (For innate immune responses, see Appendix and [57, 58]).

Although the secretion of serum IgA antibodies through bile into the gut has been observed in rodents [59], it has not been found in piglets or humans, thus limiting the significance of results obtained with rodents.

Rat

Certain rat strains develop diarrhea and systemic disease after infection with various RV strains [60–62]. The rat model has been used to determine the kinetics of viremia and the spread and pathology of RV infection in extra-intestinal organs [61].

Rabbit

Rabbits have been explored as a model of homologous and heterologous RV infection, transmission, and protection studies [63–65]. Immunity against a homologous RV strain was demonstrated after parenteral administration of inactivated virus [66]. However, the mechanisms of protection have not been studied further.

Piglet

Piglets can be infected with porcine and human RV strains, resulting in disease in both instances [67–69]. VP7- or VP4-specific antibodies protect against challenge with an antigenically cross-reactive virus [70]. The correlates of protection against challenge with human RV (G1P[8]) were found to be the presence and concentration of RV-specific IgA antibodies in serum or intestine, not the concentration of RV-neutralizing antibodies in either compartment [68, 71, 72]. VP6-specific IgA antibodies do not convey protection in this model [73].

Calf

In calves fed with colostrum containing high concentrations of antibody to bovine RV, onset of diarrhea was delayed and its severity was decreased [74]. These findings led to a strategy of vaccinating dams shortly before delivery, to boost levels of RV-specific antibody in colostrum. Success of this mode of vaccination depends on timing and dose [75, 76]. The procedure has also succeeded in horses and their foals [77]. (For innate immune responses, see Appendix and [18]).

Lamb

Gnotobiotic lambs can be infected with lamb RV strains and typically clear the virus 8–9 days after infection [78]. An increase in IL-4 production was observed in jejunal Peyer's patches at 3 days after infection, and RV-specific IgA and IgG were found in serum and nasal secretions [78]. Newborn lambs infected with bovine RV developed RV-specific T cells ($CD45^+$) at 2–3 days after infection (ie, several days before the appearance of RV-specific neutralizing antibodies in serum [79]).

IMMUNE RESPONSES IN HUMANS AFTER RV INFECTION

Humoral Immune Responses

In a seminal paper, Velazquez et al. [80] followed up 200 healthy children over their first 2 years of life for the occurrence of RV disease and for associated immune responses. Primary RV infection typically resulted in AGE, but protection developed against subsequent RV infections, with a progressively lower risk of disease. No moderate or severe disease was observed after 2 RV infections. Symptomatic and asymptomatic infections conferred similar degrees of protection [80–82], highlighting the importance of asymptomatic RV infections for RV epidemiology and protection [80, 81, 83, 84]. The frequency of asymptomatic RV infection among children prospectively observed in day care centers was found to be 3–4 times higher than that of symptomatic infections [85]. Immunity after neonatal RV infection did not confer protection from reinfection [86–88], but in some cases, it protected against severe clinical disease [86, 87].

Chiba et al. [89] presented the first evidence that protection against RV AGE can be serotype specific and related to the levels of NT antibodies against the homotypic virus. An NT antibody level $\geq 1/128$ conferred protection against RV disease. Data from Velazquez et al. [80] and others [81, 90, 91] suggest that humoral RV immunity is correlated with

Table 1. Immune responses after natural rotavirus (RV) infection

Host species	Production of RV-specific protective IgG	Production of RV-specific protective IgA[b]	RV protein specificity of protective antibodies[a]	Innate immune responses	Cell-mediated protective immune responses	Importance of α4/β7 homing receptor	References
Mouse	+	+	VP7+, VP4+, VP6+	IL-15 induction+ NKC	CD8+	+	[34–44, 46–58]
Rabbit	+		VP7+, VP4+	?	?	?	[63–65]
Piglet	+	+	VP7+, VP4+ VP6-	?	?	?	[67–70, 73]
Calf	+		VP7+, VP4+	TLR-3 induction	?	?	[18, 75, 76]
Lamb	+	+	?	IL-4 induction	CD45+	?	[78. 79]
Human	+	+	VP7+, VP4+, VP6? VP2? NSP2? NSP4?	TLR induction ?	CD8+, DCs	+	[15, 16, 26, 27, 80, 81, 84, 85, 88–99, 106–118]

NOTE. DC, dendritic cells; IL, interleukin; NKC, natural killer cells; NSP, nonstructural protein; TLR, Toll-like receptors; VP, viral protein.

[a] VP7, VP4: neutralizing; VP6, VP2, and other RV proteins: nonneutralizing.

[b] In serum or fecal samples.

protection. Homotypic and heterotypic NT antibody responses have been found in children after primary RV infection, suggesting the presence of cross-reactive NT epitopes [92]. Children with heterotypic responses tend to be older and to carry pre-existing RV-specific antibodies [93]. As technologies have developed, fecal specimens have been investigated for the presence of RV-specific IgA antibodies; at high levels, IgA antibodies correlate well with protection [94–98]. RV-specific serum IgA antibodies were shown to have NT activity, also reacting with epitopes known to elicit heterotypic protection [99].

Transplacentally acquired maternal antibodies may confer a weak protection against RV disease during the first months of life [100–102]. Breastfeeding may provide passive protection to infants [103, 104], but the significance of this mechanism for protection in humans is controversial [105].

The majority of RV-specific B cells circulating in the blood in children express the gut-specific homing receptor α4β7 [106, 107], suggesting local protective action.

Despite the importance of the RV type–specific immune responses, protection after natural RV infection is not necessarily correlated with the presence of serotype-specific NT antibodies [108]. The RV proteins VP2 and VP6 (antibodies against which are not neutralizing) carry immunodominant epitopes, and antibodies directed against them are found in most serum samples from convalescing individuals [109, 110], as are antibodies to NSP2 and NSP4 [111–113]. The clinical significance of nonneutralizing RV-specific antibodies for protection is not known.

Cellular Immune Responses

RV infection is a relatively poor inducer of cytokine-secreting, virus-specific $CD8^+$ cells [114], although these cells are present in the peripheral blood in most adults [115]. Circulating RV-specific T-helper (Th) cells are detected in blood samples from infants during the convalescent phase [26, 27]. Dendritic cells infected with RV in vitro can stimulate RV-specific T cells to secrete Th1 cytokines [116, 117] and have been shown to produce IFN-γ after infection with rhesus RV [117] but are less efficient in presenting antigens in infants and young children than in adults [114]. The role of cell-mediated immune responses for protection in humans remains to be explored.

Innate Immunity

(See Appendix and [118]).

Conclusions

From the immune responses to natural RV infection in animals and humans the following mechanisms of protection have been deduced (Table 1):

- the outer layer proteins VP7 and VP4 elicit IgG and IgA NT antibodies which correlate with protection from RV disease;
- protective efficacy of VP6-specific, non-NT IgA antibodies has been found in mice but has not been confirmed in piglets;
- RV-specific IgA antibodies in the gut lumen are associated with protection, although NT activity has been proven for only a few of them;
- cell-mediated, RV-specific immune responses have been observed after RV infection and, in some instances, have been found to be associated with protection;
- innate immune responses have been recorded after RV infection, but their significance for protection is not clear;
- B cells carrying the α4β7 instestinal homing receptor are important for clearance of RV infection and protection from reinfection
- protection against RV disease is not always correlated with the presence of type-specific or cross-reactive NT antibodies, but the significance of non-NT antibodies for protection against RV disease is not clear; and
- in general, data on protection against RV disease in animals are of limited significance for humans because of differences in gut physiology, the inbred nature of many experimental animals, and the experimental precondition of animals being RV naive.

IMMUNE RESPONSES AND PROTECTION AFTER VACCINATION IN ANIMALS

Mouse

In mice, levels of intestinal and serum RV-specific IgA and the degree of protection are correlated [40, 119, 120]. Intramuscular inoculation of mice with murine RV strains induces complete protection from RV shedding [121]. Vaccination of mice with NSP4 was shown to induce partial immunity against RV [122]. High levels of maternal RV-specific antibodies in newborn mice impair their response to vaccination with RV-like particles [123].

Piglet

In piglets, a direct correlation between the level of RV-specific IgA-secreting cells, levels of serum and gut IgA and the degree of protection has been found [72, 124]. Oral vaccination of piglets with an attenuated human RV vaccine conferred protection that could be augmented by a booster with VP 2/6 virus–like particles (VLPs) [125] and was correlated with immune responses to VP4 and VP7 [71, 126]. NSP4 as a vaccine has been found to be ineffective in gnotobiotic piglets [127]. Maternally derived RV-specific antibodies confer protection to piglets against natural infection during the first 2 weeks of life [128] but can interfere with the degree of protection achieved after vaccination [129, 130].

Rabbit

Broad heterotypic protection against RV challenge was induced in rabbits by parenterally administered inactivated RV particles or VLPs [66, 131].

Baboon

Vaccination of pregnant baboons, followed by booster vaccinations at 1–2 and 14 weeks after delivery, significantly increased RV-specific IgG and NT antibody concentrations in serum and protected the offspring from RV infection [132]. Concomitant increases in RV-specific IgG, IgA, and NT antibodies were observed in milk, leading to the hypothesis that protection conferred by milk may be mediated by IgA antibodies [132].

PROTECTION AFTER VACCINATION IN HUMANS

RV Vaccination: Different Concepts

Heterologous immunity has been demonstrated in gnotobiotic calves developing resistance against a human RV strain after vaccination with a calf RV strain [133]. This observation, together with data from gnotobiotic piglets [134], formed the basis for the use of bovine RV 4237 (BRV, G6P6[1]) as the first human RV vaccine candidate [135]. Although this virus did not share NT epitopes with the most frequently cocirculating human RV strains, it induced heterotypic protection [136]. After the recognition that levels of NT antibodies against RV do not represent the only correlate of protection, different concepts of vaccine development emerged [28–33]. Multivalent vaccines are required if the production of strong NT antibody responses is the goal. Those would contain virus strains (mostly reassortants) carrying VP7 and VP4 molecules representative of the main cocirculating wild-type human RV strains. The vaccines RotaShield (Wyeth-Lederle Vaccines) and RotaTeq (Merck) are based on this principle, the latter containing proteins of the serotypes G1, G2, G3, G4 and P1A (genotype [8]) and the RNAs encoding them. By contrast, the monovalent vaccine of serotype G1P1A[8] (Rotarix; GlaxoSmithKline Biologicals), derived from a clinical human isolate (89-12) obtained in 1989, was produced on the basis of the observation that cross-protection (ie, a heterologous immune response) develops during the course of successive natural infections [80]. RV disease can be prevented by repeated natural RV infections and by repeated vaccination with a single RV serotype. All 3 aforementioned vaccines contain live attenuated virus strains (Appendix)[137, 138]. Rotashield was discontinued in 1999 (because of epidemiological association with gut intussusception [139]). RotaTeq and Rotarix have been licensed since 2006 in numerous countries after they were found not to be associated with increased rates of intussusception in extensive phase III safety clinical trials [31, 32, 140]. These 2 vaccines are currently being applied in millions of doses, in some countries as part of universal mass vaccination programs of childhood vaccination schemes. Several updates on the development of RV vaccines have been published [141–150].

Humoral Immunity

After encouraging pilot studies (Appendix) [151–153], good protection rates were achieved with both monovalent (Rotarix) and polyvalent (RotaTeq and RotaShield) vaccines, particularly against severe disease requiring hospitalization [31, 32]. The monovalent vaccine was found to be effective over at least 2 RV seasons [30, 154]. Homotypic and heterotypic immunity has been observed with both monovalent and polyvalent vaccines [155, 156], and protection mostly but not always correlated with levels of RV type–specific IgG or IgA antibodies [28, 157–161]; protection was less correlated with the levels of RV-specific NT antibodies [108, 162, 163]. The G type–specific NT antibody responses to the multivalent RotaTeq vaccine are too low to account for the degree of protection achieved [162]. Individuals with selective IgA-D deficiency may be protected from severe RV disease by developing compensatory RV-specific IgG responses that are higher than those in IgA-competent persons [164]. Similar to mice [45, 46], NT activity was found in human serum RV-specific IgA antibodies, also reactive with VP4- and VP7-specific epitopes

Table 2. Immune responses after rotavirus (RV) vaccination

Host species	Production of RV-specific protective		RV immunogens correlated with protection	Innate immune responses	Cell-mediated immune responses	Importance of α4/β7 homing receptor	References
	IgG	IgA[a]					
Mouse	+	+	VP7+, VP4+, VP6+, NSP4+	IL-15 induction	+ T cells		[35–37, 40, 48, 57, 119–123, 172, 175–182]
	Vaccination during pregnancy[b]				+		
Rabbit	+		inact RV, RVLPs	?	?	?	[66, 131]
Piglet	+	+					
	Het. prot. Vaccination during pregnancy [b]		VP7+, VP4+, VP6-, NSP4 - RVLPs	?	?	?	[71–73, 124–130]
Calf	+	+					
	Het. prot. vaccination during pregnancy[b]						[76, 133]
Baboon	+	+ milk	RV particles				
	Vaccination during pregnancy[b]						[132]
Human	+	+	live att. RV VP7, VP4 VP6?, VP2?	?	+T cells, DCs	+	[28–32, 80, 110, 116, 135, 136, 138, 141–152, 154–165]
	Het. prot. No corr with NT abs						

NOTE. Ab, antibody; att, attenuated; corr, correlation;, DC, dendritic cell; het, heterotypic; inact, inactivated; mat, maternal; NT, neutralizing; prot, protection; RVLP, RV-like particle.

[a] Serum and fecal samples.

[b] Maternal antibodies interfering.

known to elicit heterotypic immunity [99]. Levels of RV-specific plasma IgA and RV-specific B cells carrying the gut homing receptor represented a possible, albeit weak, correlate of protection in vaccinated children [165].

At the population level, universal mass vaccination in the United States with RotaTeq delayed the start of the 2007–2008 RV season by 2–4 months, and the peak, when it occurred, was significantly lower than that during previous seasons [166]. Similar results have been obtained with Rotarix in other countries (references available upon request). The nutritional status of infants does not affect the efficacy of the RV vaccine [167]. Phase III trials in Malawi and South Africa with Rotarix have demonstrated that protection from severe RV-associated AGE is lower in South Africa (70%) and Malawi (50%; mean, 60%) [149, 150]. With RotaTeq, the protection rate was 64% in Africa and 51% in Asia [168]. Because of the high RV disease–associated mortality in these regions, a significant benefit in terms of deaths prevented, even at lower vaccine efficacy, is apparent [149, 150], because ''vaccine efficacy estimates correlate inversely with disease incidence and child mortality strata'' [168, page 518]. On the basis of these findings, the World Health Organization Strategic Advisory Group of Experts on Immunization has recommended the use of RV vaccination worldwide in all national immunization program if it can be funded and organized [150, 168].

Cell-mediated Immunity

There is very little information available regarding cell-mediated immunity after RV vaccination in humans. RV-seropositive individuals have circulating dendritic cells that are able to stimulate RV-specific T cells to produce Th1 cytokines [116]. Children with severe combined immunodeficiency may develop AGE after RV vaccination [169].

Conclusions

From RV vaccination studies in animals and humans (Table 2), the following mechanisms of protection have been delineated:

- there is a good correlation of protection with the levels of RV-specific IgA antibodies in the gut lumen;
- there is ample evidence for heterologous protection not depending on the levels of RV type–specific NT antibodies;
- RV-specific maternal antibody may interfere with the efficacy of RV vaccination in infants and young animals;
- RV-specific B cells carrying the α4β7 intestinal homing receptor are important for protection;
- monovalent and multivalent live attenuated RV vaccines are both efficacious in eliciting protection against natural RV disease in infants and young children; and
- because RV vaccination studies permit better control and analysis of data, heterotypic protection has been recognized as a considerable component of the efficacy of RV vaccines; the mechanism(s) of action remain to be clarified.

ALTERNATIVE VACCINES

(See Appendix and [170–182]).

DISCUSSION AND FUTURE STUDIES

In general, RV diarrhea is considered to be a vaccine-preventable disease [183]. However, despite the availability of 2 highly efficacious and well-tolerated RV vaccines, there are considerable gaps in the understanding of correlates of protection in humans [141, 184, 185]. In animal models, humoral immunity has been shown to be associated with protection against RV, mediated by both neutralizing and nonneutralizing antibodies. It is uncertain to what extent this may apply to humans. Although the efficacy of RV-specific $CD8^+$ cells in eliminating RV infection has been demonstrated in animals, their contribution to protection in humans is still being explored. The same can be said of the significance of innate immunity for protection against RV disease, particularly in humans.

The most widely accepted correlate of protection from RV AGE is the presence of RV-specific IgA antibodies in the gut (copro-antibodies). Only high titers of RV-specific copro-IgA antibodies are correlated with protection. Most intestinal IgA antibodies appear to be directed against VP6 (ie, they are not neutralizing). Mouse VP4-specific and human serum VP4- and VP7-specific IgA antibodies neutralize RV in vitro. More work should be devoted to testing NT activity of RV-specific copro-IgA antibodies in humans. Protection may also be mediated by RV-specific IgG antibodies diffusing between enterocytes with damaged intercellular bridges.

Although the 2 currently licensed, live attenuated RV vaccines differ in composition and their proposed mechanisms of protection, they are both highly efficacious in preventing severe RV disease and hospitalization against diverse RV types. This astonishing fact is not fully understood.

Homotypic and heterotypic immunity has been observed worldwide after RV vaccination. The extent to which existing RV vaccines exert heterotypic protection, particularly in developing countries, is beginning to be explored. Because improvements in drinking water supplies and sanitation have not significantly decreased the prevalence of RV disease [186], vaccination is considered to be the most effective public health strategy to prevent RV AGE and to reduce disease burden. The acceptance of RV vaccination as a universal mass vaccination approach and the degree of uptake of vaccination in different settings will be critical for the development of herd immunity, which has possibly started in the United States [187] but requires further investigation. Postmarketing surveillance over several seasons of RV vaccination programs will reveal the extent to which heterotypic protection affects wild-type RV strain diversity and whether variations in RV strain diversity are attributable to the natural fluctuation or represent true escape mutants [188, 189]. At present, it is too early to determine whether worldwide RV vaccination programs will lead to worldwide prevention of severe RV disease [190, 191].

There is an urgent need for further research into the mechanism(s) and correlates of protection of RV-specific immune responses in humans that may be acquired transplacentally or through natural infection, vaccination, or breastfeeding. Such investigations may also help to comprehend better the immunology of other enteropathic viruses, particularly in young children.

Appendix

Innate Immune Responses

Genomic rotavirus dsRNA and a synthetic analogue, polyinosinic-polycytidylic acid have been shown to induce intestinal injury in mice by stimulating intestinal intraepithelial lymphocytes (IILs) to produce high levels of interleukin (IL)-15 [57], suggesting that toll-like receptor 3 (TLR3) present on IILs may be involved in RV pathogenesis. Natural killer (NK) cells were found to be up-regulated via IL-15 induction after exposure to different RV strains in vitro [58], suggesting innate immune surveillance of RV infection.

Calves infected with bovine RV produce a strong innate immune response, as measured by TLR3 increase, but the IFN and pro-inflammatory cytokine–associated pathways are down-regulated [18].

There are few data available with respect to innate immunity in humans after natural RV infection. An increase in the expression of 5 TLRs has been found in children after acute RV infection [118].

Further Details on Human RV Vaccines

Rhesus RV (RRV, type G3P5B[3]) served as the genetic backbone in RotaShield and the bovine RV WC3 (type G6P7[5]) in the RotaTeq vaccine. Although the WC3 RV in RotaTeq can be regarded as attenuated in the human host, it underwent 15 passages in African green monkey kidney (CV-1) cells [137], and the 89-12 human RV in Rotarix was attenuated by >40 passages in Vero cells [138]. There are some differences in growth in the human host (the 89-12-derived RV is shed to higher percentage than WC3-derived RVs), but both vaccines are highly efficacious in protecting against severe RV disease after infection with different RV strains [31–33].

Vaccination of children with the RRV(G3)-based vaccine was shown to provide type-specific protection [151]. The BRV- and RRV-based vaccines protected against heterotypic wild-type human RV strains [152]. Vaccination of adults with the human–bovine (WC3) reassortant RV vaccine resulted in the production of serum and local (copro-) IgA antibodies [153].

In general, protection from severe RV disease by vaccination is higher than

protection from any RV infection [28–32], and therefore, severe AGE has been chosen as the primary efficacy end point [29].

Alternative Vaccines

All currently licensed RV vaccines contain live attenuated RV strains of various combinations of G and P types. Another live attenuated RV vaccine, based on mono-reassortants of the bovine UK Compton RV strain carrying human RV G and P type proteins, is in an advanced state of clinical trials [152, 170]. A neonatal G3P[6] human RV vaccine has demonstrated some cross-protection against G1P[8] wild-type RV [86], but in a phase II clinical trial, only moderate efficacy was observed [171].

Other virus-derived preparations have been considered for vaccination of humans: killed whole virus, isolated viral proteins (VP7, VP4, VP6, NSP4), virus-specific peptides, virus-like particles, DNA-based vaccines, and edible vaccines (expression of RV-specific proteins in plants) [69, 147, 172, 173]. Mice have been protected by RV VP4 expressed from *Lactobacillus casei* [174]. None of these candidate vaccines has, to date, progressed to clinical trials.

RV proteins other than those on the surface of particles have been shown to be correlated with protection. Nonreplicating VLPs, consisting of VP2/6 (double-layered particles) or VP2/6/7 (triple layered particles), are immunogenic [131, 172, 175–180]. Natural RV infection and RV vaccinatin in humans result in the production of high levels of VP2- and VP6-specific antibodies [110, 181]. Protection with VP2/6 double-layered VLP constructs has been achieved in the mouse model [172, 175, 176, 182] but not in the piglet model [73]. VP2/6 VLPs have been used as a boosting antigen after primary vaccination with an attenuated vaccine [125].

Supplementary Data

References (50 selected references, with numbers in Text maintained. The full list of references is in the supplementary data are available at http://www.oxfordjournals.org/our_journals/jid/online).

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