Rotavirus vaccines: recent developments and future considerations

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Abstract | Two new vaccines have recently been shown to be safe and effective in protecting young children against severe rotavirus gastroenteritis. Although both vaccines are now marketed worldwide, it is likely that improvements to these vaccines and/or the development of future generations of rotavirus vaccines will be desirable. This Review addresses recent advances in our knowledge of rotavirus, the host immune response to rotavirus infection and the efficacy and safety of the new vaccines that will be helpful for improving the existing rotavirus vaccines, or developing new rotavirus vaccines in the future.

Host-range restriction
(HRR). The limited capacity of certain viruses to grow and transmit efficiently in an animal species that is distinct (heterologous) from the animal species they naturally infect (homologous).

Intussusception
A pathological event in which the intestine acutely invaginates upon itself and becomes obstructed, followed by local necrosis of gut tissue.

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Natural rotavirus infection efficiently protects against severe disease associated with re-infection[1]. Two virus surface proteins, VP4 and VP7, are targets of neutralizing antibodies and either antibody can mediate protection[2]. Both proteins are found in various conformations, which form the basis for a binary serological classification scheme[3] (BOX 1). Rotaviruses generally exhibit substantial host-range restriction (HRR), such that most animal rotaviruses are highly attenuated in 'heterologous' human hosts and vice versa. On the basis of these simple facts, a human–simian reassortant rotavirus vaccine (RotaShield) containing four serotypically distinct VP7 components was developed in the 1990s, and was shown to be safe and effective in preventing severe rotavirus diarrhoea in young children in the United States and Venezuela[4]. This vaccine was assumed to be attenuated because most of its genome was derived from a heterologous simian host.

RotaShield was licensed in the United States in 1998 and was given to almost 1 million children before a temporal association between vaccine administration and gut intussusception was detected[5]. For this reason, RotaShield was withdrawn from the market and this removal created a pressing need for the development of new, safer rotavirus vaccines. Two new vaccines (Rotarix from GlaxoSmithKline and RotaTeq from Merck) have recently been developed. To address concerns about safety, large Phase III clinical trials were undertaken for both vaccines, each involving more than 60,000 infants[6,7]. Both vaccines were shown to be safe, were not associated with intussusception, and provided >70% and 90% protection against any rotavirus diarrhoea and severe rotavirus diarrhoea, respectively[6,7]. Importantly, both vaccines reduced the rates of gastroenteritis-related hospitalization from any cause by more than 40%, suggesting that the real incidence of rotavirus disease could have been underestimated, or that the vaccines might provide non-specific protection against other enteric pathogens. Although both vaccines are being licensed in an increasing number of countries worldwide, the mechanisms by which they induce protection and the molecular basis of their attenuation are not well understood and some issues concerning their safety and efficacy remain to be clarified.

Several summaries[8,9] of rotavirus vaccines have been published. In this Review, we discuss recent advances that might be helpful for improving the current rotavirus vaccines or are relevant to the development of new rotavirus vaccines in the future, with an emphasis on the immunology and mechanisms of protection that are induced by the two new vaccines, as well as other related issues not addressed in our recent review of rotavirus vaccine-induced immunity[2].

Rotavirus

Rotaviruses belong to the family *Reoviridae*, which are non-enveloped, icosahedral viruses with an 11-segment double-stranded RNA genome[3,10]. There are six rotavirus structural proteins, which form three concentric layers (FIG. 1). The internal layer, or core, surrounds the viral genome, and contains the scaffolding protein VP2, the RNA-dependent RNA polymerase VP1, and VP3 (a guanylyltransferase and methylase) (TABLE 1). The intermediate layer is made of VP6, the major structural protein.

Box 1 | **Rotavirus classification**

Rotaviruses are classified in groups (A–E) and subgroups on the basis of the antigenic specificity of the major virus structural protein VP6 (REF. 3). Rotaviruses from group A are the most common cause of human disease and, unless specified, only group A rotaviruses are discussed in this article. Rotaviruses are further classified into serotypes on the basis of the neutralizing epitopes of VP7 (a glycoprotein), called G serotypes, and VP4 (protease-sensitive), called P serotypes. As the viral genome is segmented, the genes encoding VP7 and VP4 can segregate independently, generating a binary nomenclature. There are 15 G serotypes, which are generally equivalent to the G genotypes (determined by sequence relatedness), and commonly either the G serotype or G genotype is reported. More than 90% of human rotavirus strains identified globally are classified as G1, G2, G3, G4 or G9 strains. There are 14 P serotypes and at least 25 P genotypes (designated in brackets), which are not always equivalent, so both are generally reported; for example, the Wa strain of rotavirus that is frequently used in laboratories is referred to as a P1A[8]G1 virus. In humans, G1, G3, G4 and G9 are frequently associated with P1A[8] and G2 with P1B[4], limiting viral diversity. Therefore, the majority of circulating rotaviruses worldwide share crossreactive neutralizing epitopes of the P1 serotype.

The external layer is made up of VP7 and is decorated with spikes of VP4 (REFS 3,10). In infected cells, six non-structural proteins (NSP1–6) are produced (TABLE 1). Crystallographic studies of several of these proteins have been carried out and their functions are partially known (TABLE 1). To be fully infectious, VP4 must be cleaved by an intestinal lumen protease (trypsin), with the consequent formation of VP5* and VP8* (TABLE 1), which interact with cellular receptors.

The biology and molecular characteristics of rotaviruses have been studied by many investigators over the past 30 years. However, research on rotavirus has lagged behind that of other viruses because of the absence of tractable reverse-genetics systems, which can be used to manipulate the viral genome directly. For this reason, in order to study viral gene function and isolate rotaviruses with selected properties of interest, investigators have taken advantage of the fact that, when two rotaviruses co-infect the same cell (both *in vitro* and *in vivo*), they undergo gene reassortment at high frequency, creating progeny viruses with an assortment of genes from both parental strains[3]. Such gene reassortment was used to create the RotaShield and RotaTeq vaccines, both of which were developed based on the hypothesis that vaccines derived from animal hosts will be attenuated in humans by HRR.

Recently, two powerful new approaches have become available for the study of rotaviruses. *In vitro* studies using small interfering RNAs have begun to fill many gaps in our knowledge about the function of specific viral genes[11–14]. In addition, the first reverse-genetics method to create rotaviruses with complementary DNA (cDNA)-derived reassorted genes has become available, which will enhance our ability to study the role of the different rotavirus proteins in viral morphogenesis, pathogenesis and immunity *in vivo*[15].

Reverse genetics
A method that allows the production of viruses that possess genes derived from cloned cDNA.

Small interfering RNAs
Small antisense RNAs (20–25 nucleotides long) that are generated from specific double-stranded RNAs that trigger RNA interference.

Burden of disease and epidemiology

Every year, rotaviruses cause approximately 111 million episodes of gastroenteritis that require home care, 25 million clinic visits, 2 million hospitalizations and ~611,000 (range 454,000–705,000) rotavirus-related deaths in children younger than 5 years of age worldwide[16,17]. The burden of disease is unevenly distributed between developed and developing countries, probably for socioeconomic and epidemiological reasons, with the majority of deaths occurring in the developing countries. However, the burden of disease in developed countries is also significant. For example, in the United States, it is estimated that rotavirus is associated with 4–5% of all childhood hospitalizations, and between 1 in 67 and 1 in 85 children will be hospitalized with rotavirus-mediated gastroenteritis by 5 years of age[18]. This rate has not declined between 1993 and 2002 (REF. 19). In this context, it is not surprising that rotavirus vaccines are considered a cost-effective intervention in the United States[20].

The epidemiology of rotavirus is a complex, changing phenomenon[21]. The geographical distribution of different human rotavirus strains varies; P1A[8]G1 viruses are more frequent in North America, Europe and Australia than in South America, Asia and Africa[21] and have been the most frequently encountered viruses during the past 30 years. In some areas of India, Brazil and Africa, P[6]G9, G5 and G8 rotaviruses, respectively, are more frequent than elsewhere[21]. One possible explanation for this is that there is more reassortment between human and animal rotaviruses[22]. There is increasing evidence for zoonotic transmission of animal rotaviruses to humans, leading either to animal rotaviruses causing infection or disease directly, or to reassortment of one or more of their genome segments into rotaviruses circulating in humans[23,24].

Along with geographical variations, temporal variations in rotavirus distribution are also important. Most epidemiological studies up to the early 1990s showed a predominance of G1–G4 strains but since then, P[8]G9 or P[6]G9 strains have emerged worldwide and G9 rotavirus accounted for 4.1% of all isolates in recent studies[21]. New G12 (REFS 25,26) strains, detected recently in India with increasing frequency, could represent the next emerging rotavirus genotype and could be a potential challenge to the present, and any future, vaccines.

Rotavirus infection tends to occur year-round in many tropical countries, whereas seasonal winter epidemics occur in most countries with temperate climates[3]. Outbreaks of rotavirus that can affect both adults and children are relatively rare[27]. It is important to bear in mind that group B rotavirus (which in the past was mainly detected in adults with gastroenteritis in China, India and Bangladesh) have recently been shown to be associated with up to 18.5% of episodes of gastroenteritis in children whose samples did not seem to contain group A rotavirus[28].

CD8⁺ T cells
A subpopulation of T cells that express the CD8 receptor. CD8⁺ cells recognize antigens that are presented on the surface of host cells by major histocompatibility complex (MHC) class I molecules, leading to their destruction, and are therefore also known as cytotoxic T cells.

CD4⁺ T cells
A subpopulation of T cells that express the CD4 receptor. CD4⁺ cells recognize antigens that are presented on the surface of host cells by major histocompatibility complex (MHC) class II molecules. These cells aid in immune responses and are therefore referred to as T-helper cells.

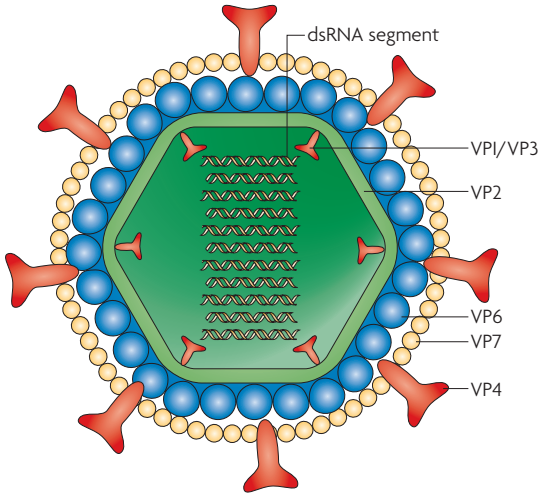

Figure 1 | **Rotavirus.** The figure shows a schematic representation of a rotavirus virion.

Rotavirus immunity

Immunity in animal models. Animal models have been useful in improving our understanding of immunity to rotavirus[2] (FIG. 2). In adult mice (the model that is best suited for addressing mechanistic questions of immunity), after infection with a homologous murine rotavirus, CD8⁺ T cells have a role in the timely resolution of primary infection, and CD4⁺ T cells have an important, but not essential, role in the generation of rotavirus-specific intestinal immunoglobulin A (IgA), which is the principal effector of long-term protection against rotavirus infection[29]. As would be expected by the fact that rotavirus infection includes a viraemic phase[30,31], both intestinal and systemic rotavirus-specific B-cell responses are observed in mice[2]. However, only rotavirus-specific plasma cells that reside in the intestine seem to have an antiviral effect, suggesting that mucosal, but not systemic, antibodies provide protection in this model[2,32,33]. The relevance of these findings to immunity in vaccinated children remains to be determined.

Recent studies have extended our knowledge of the T-cell response and immunity to rotavirus in neonatal mice[34–36]. On the one hand, the CD4⁺ and CD8⁺ T-cell response of mouse pups to homologous murine rotavirus appears to be weak, especially for CD4⁺ T cells[34]. On the other hand, neonatal mice show reduced protection against rotavirus after intranasal vaccination with recombinant VP6, a vaccine that specifically requires the induction of T cells[35]. The lack of protection of newborn pigs with a similar VP6 vaccine[37] that efficiently protects adult mice could, in part, be due to the immaturity of their immune system. Notably, an oral heterologous rhesus rotavirus (RRV) vaccine induced lower levels of neutralizing antibodies in neonatal mice than in adult mice, and the murine rotavirus did not induce any neutralizing antibodies[35]. Thus, as with the CD4⁺ T-cell response described above, the induction of neutralizing antibodies in neonates after infection with homologous rotavirus is relatively weak. Because in rodents, the heterologous RRV replicates more efficiently at systemic sites than homologous murine rotavirus[30,38,39], it is possible that the lower immunogenicity of the homologous rotavirus, relative to the heterologous rotavirus, is due to its preferential localization in the tolerogenic environment of the intestine[40].

It should be mentioned that rotavirus infection in animals and humans has been linked to autoimmune diseases by some investigators. Clinical data, and studies in mice, have suggested a role for rotavirus in the pathogenesis of diabetes, but recent studies in mice and children do not support these findings[41,42]. Some groups[43], but not others[44], have found group C rotavirus in children

Table 1 | **Characteristics and known functions of rotavirus proteins**

Protein	Function
VP1	RNA-dependent RNA polymerase; ssRNA binding; located at the five–fold axis inside the inner capsid; forms a transcription complex with VP3.
VP2	Inner capsid structural (core) protein; non-sequence-specific RNA-binding activity; required for replicase activity of VP1.
VP3	Guanylyltransferase and methyltransferase; part of the virion transcription complex with VP1.
VP4	Trimers of VP4 form the outer capsid spike; P-type-specific neutralization antigen; virulence determinant; haemagglutinin; cell-attachment protein; cleavage by trypsin into VP5* and VP8* enhances infectivity.
NSP1	Associates with the cytoskeleton; extensive sequence diversity between strains; has a role in suppressing the host IFN-α response; non-essential in some strains.
VP6	Major virion protein; middle capsid structural protein; homotrimeric structure; subgroup antigen; required for transcription.
NSP3	Homodimer; specifically binds 3′-end of rotavirus mRNA; binds elongation factor eIF4G1; involved in translational regulation.
NSP2	NTPase and helicase; non-specific ssRNA binding; involved in viroplasm formation; binds NSP5 and VP1; essential for dsRNA synthesis.
VP7	Outer capsid structural glycoprotein; G-type neutralization antigen; RER transmembrane calcium-binding protein.
NSP4	Viral enterotoxin; receptor for budding of double-layered particles through the ER membrane; glycoprotein; modulates intracellular calcium levels and RNA replication; secreted cleavage product.
NSP5	Interacts with NSP2 and NSP6; forms homomultimers; *O*-linked glycosylation; hyperphosphorylated; binds ssRNA; component of viroplasm; essential for viral replication.
NSP6	Product of the second out-of-frame open-reading frame of gene segment II; interacts with NSP5; localizes to the viroplasm.

ds, double-stranded; ER, endoplasmic reticulum; IFN, interferon; RER, rough endoplasmic reticulum; ss, single-stranded.

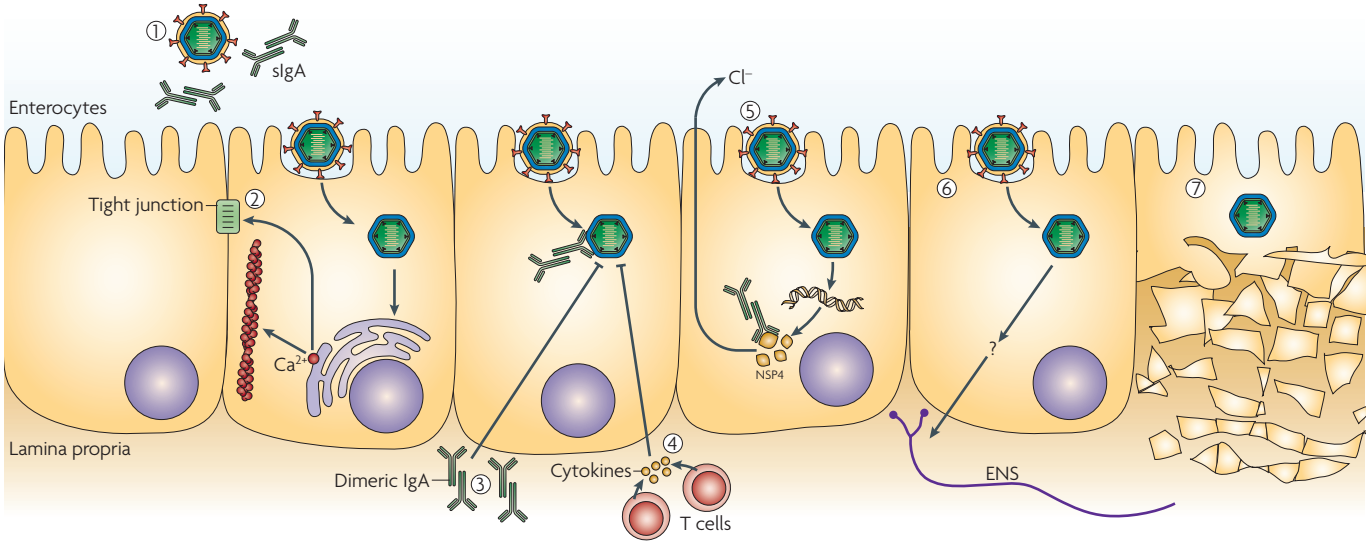

Figure 2 | **Potential mechanisms of rotavirus pathogenesis and immunity.** The mechanisms of rotavirus pathogenesis and immunity are not completely understood and vary depending on the animal species studied[3,10]. A summary of the potential mechanisms of rotavirus pathogenesis and immunity, mostly (steps 3 to 5 in particular) derived from observations in rodents is shown. In step 1, neutralizing antibodies directed against VP4 and/or VP7 can prevent viral binding and penetration, inducing viral exclusion. If this mechanism fails, as shown in step 2, rotavirus replication inside enterocytes causes altered metabolism of enterocyte membrane proteins inducing malabsorptive or osmotic diarrhoea. Rotavirus also increases the concentration of intracellular calcium, which disrupts the cytoskeleton and the tight junctions, raising the paracellular permeability. During step 3, intracellular viral replication can be inhibited by secretory anti-VP6 immunoglobulin A (IgA) during transcytosis across enterocytes. In step 4, cytokine-secreting rotavirus-specific T cells can also inhibit viral replication. If viral replication is not stopped, as shown in step 5, replicating rotavirus produces non-structural protein 4 (NSP4), a toxin which induces a secretory non-cystic fibrosis transmembrane conductance regulator (CFTR)-mediated diarrhoea. By an unknown mechanism (suggested by some investigators to be dependent on NSP4) rotavirus can also stimulate the enteric nervous system (ENS) (as shown in step 6), inducing secretory diarrhoea and increasing intestinal motility. Drugs that inhibit the ENS are useful in treating rotavirus diarrhoea in children. Antibodies against NSP4 could potentially have an effect against the last two mechanisms. Late in the infectious process, rotavirus kills the host cell (as shown in step 7), further contributing to malabsorptive or osmotic diarrhoea. Despite its 'enteric nature', rotavirus antigens, double-stranded RNA and infectious particles have been found in the blood of children and systemic organs in animals[31]. The role of these systemic antigens and/or virus in the pathogenesis of rotavirus-induced disease is currently unknown. sIgA, secretory IgA.

with extra hepatic biliary atresia (EHBA), and evidence in the murine model has clearly shown a role for rotavirus in this disease. For example, adoptive transfer of T cells from mice with RRV-induced EHBA into naive syngeneic severe combined immune deficient (SCID) recipient mice resulted in bile-duct-specific inflammation in the absence of detectable virus[45]. Notably, it seems that simian rotaviruses, but not murine or human strains, induce EHBA in mice[46]. Finally, there is some preliminary evidence that associates rotavirus infection with coeliac disease[47,48] and which requires further research.

Immunity in humans. Studies of infants with natural rotavirus infection are crucial to our understanding of human immunity to rotavirus (BOX 2; FIG. 2). In agreement with the animal studies, the levels of rotavirus-specific serum IgA measured shortly after natural infection in children generally correlate with intestinal IgA levels, and in many, but not all studies, the serum IgA level provides a good correlate of protection[2]. Furthermore, T-cell responses to rotavirus are related to the development of protective antibodies[49].

Studies of the human rotavirus-specific T-cell response, using an intracellular cytokine flow-cytometry assay[50] and ELISPOT[51], showed that both healthy and rotavirus-infected adults have relatively low frequencies of $CD4^+$ and $CD8^+$ rotavirus-specific T cells that secrete interferon (IFN)-γ, but not interleukin (IL)-13 or IL-4 (REFS 50,51). In children with rotavirus gastroenteritis, the number of these cells is low or undetectable[50,51]. Consequently, the pattern of cytokines that are secreted by rotavirus-specific $CD4^+$ T cells in children is not clear, but it could be a mixed T-helper 1 (T_H1) and T_H2 pattern, as found in neonatal pigs[52].

Supporting the finding of a weak T-cell response to rotavirus, studies that tried (but failed) to identify an association between rotavirus infection and type 1 diabetes have shown that proliferative T-cell responses to rotavirus in prospectively followed rotavirus-infected children are transient[53], are present in only a minority of healthy 3–7-year-old children (35%), and are relatively lower than the responses to several other antigens. A recent study that compared the patterns of gene expression in peripheral blood mononuclear cells (PBMCs)

Extra hepatic biliary atresia
A disease of infancy that is characterized by inflammation and fibrosis of the extrahepatic biliary tract, resulting in cirrhosis.

ELISPOT
An enzyme-linked immunoassay to identify individual cells that secrete a particular molecule.

Antigenaemia
The presence of viral antigens in the blood.

from children with rotavirus diarrhoea and healthy children showed that the first group had increased expression of genes involved in B-cell differentiation, maturation, activation and survival, but lower levels of mRNA for genes involved in the various stages of T-cell development. Importantly, this study also demonstrated a reduction in the total lymphocyte population and in the proportions of $CD4^+$ and $CD8^+$ T cells in PBMCs[54], suggesting that rotavirus alters T-cell homeostasis.

The T-cell response to rotavirus in humans therefore seems to share characteristics with the T-cell response that is seen in animal models, being transient and of low intensity, especially in rotavirus-infected children[50]. However, the response appears to be more robust in healthy adults, as demonstrated by a recent study that evaluated T-cell responses to multiple rotavirus antigens by ELISPOT[55].

The recently licensed rotavirus vaccines

The rotavirus vaccine field advanced significantly in 2004 when the Rotarix vaccine was approved for use in Mexico and subsequently in other Latin American and European countries. Further progress was made in 2006 when the US Food & Drug Administration (FDA) approved the bovine–human reassortant vaccine RotaTeq for use in the United States. These vaccines have been designed using different approaches (FIG. 3).

Clarification of the mechanisms of rotavirus-induced gastroenteritis and the immune response to rotavirus infection (FIG. 2), and complementary studies to identify the molecular basis of viral virulence, attenuation and HRR, are important areas that must be addressed before the current vaccines can be improved. Analysis of the genome sequence of the attenuated human Rotarix vaccine strain and its virulent wild-type parent strain seems necessary to identify the mutations that are responsible for attenuation. It would not be expected to present a major problem if this P1A[8]G1 rotavirus vaccine strain were to revert to its original phenotype; at the most, it would mean that another virulent P1A[8]G1 rotavirus would be introduced into the community, joining many other wild-type P1A[8]G1 'natural' rotaviruses. However, this vaccine is shed in moderate quantities by vaccinees and the basis for its genetic stability is not yet defined. In addition to identifying the genetic basis for attenuation, the degree of transmissibility and the potential routes (enteric and respiratory[56]) by which this vaccine strain can spread in humans or other susceptible hosts need further investigation. The potential beneficial consequences of the spread of the vaccine strain in the human population, such as 'herd immunity', could be addressed in post-licensure surveillance studies. Moreover, a clearer understanding of the genes that determine HRR for RotaTeq (the genetic backbone of which is derived from a bovine rotavirus) is warranted, although many assume that the basis for HRR is multigenic, and such rotavirus vaccines seem unlikely to revert to virulence during replication in the heterologous human host. Finally, for both vaccines, it would be interesting to determine whether antigenaemia and viraemia (events observed during natural rotavirus infection[57,58]) occur after vaccination, although it is currently unclear whether viraemia has any significant pathological consequences during wild-type infection.

RotaTeq. This vaccine is composed of five reassortant rotavirus strains, each of which was derived from a parental WC3 bovine strain and each of which contains a gene encoding VP4 or VP7 from a rotavirus of human origin. Hence, this vaccine was formulated to contain most of the different serotypes that a child will be exposed to, on the basis of the assumption that this approach is the most effective way to induce broad protective immunity. The human VP7 or VP4 represent the most common circulating human rotavirus serotypes (G1, G2, G3, G4 and P1A[8])[59]. Although it is generally referred to as a pentavalent vaccine, RotaTeq actually contains seven neutralizing determinants, because it

Box 2 | The main features of natural human immunity to rotavirus

Infants and young children have multiple rotavirus infections in the first few years of life. The first infections, which are the most severe, generally occur at 3–12 months of age, when circulating maternal rotavirus-specific immunoglobulin G (IgG) is waning. However, differences between countries appear to be important, with children from less developed countries having a higher risk of early infection. In children from countries with 'intermediate' levels of development, up to 50% of primary infections occur during the second year of life. In these countries it has been documented that more than 50% of first rotavirus infections are asymptomatic. The severity of disease decreases with subsequent infections, and symptomatic and asymptomatic infections are equally effective at stimulating protective immunity. Sterilizing immunity has not been reported, but the occurrence of two infections, whether symptomatic or asymptomatic, results in virtually complete protection against moderate-to-severe gastroenteritis and a single natural rotavirus infection is roughly comparable to the currently licensed vaccines in preventing subsequent severe disease. Consequently, a reasonable expectation for a live rotavirus vaccine is to protect against severe disease but not against infection.

The mechanism of immune protection after natural infection is unclear. Rotaviruses induce a mucosal intestinal IgA response which, for unknown reasons, is not generally persistent. Increased titres of serum rotavirus-specific IgM, IgG and IgA are also observed after infection. Some of the serum IgA has a secretory component, suggesting that it is derived from 'spillover' from the intestine. This might explain why serum rotavirus-specific IgA levels provide a good correlate of protection against disease. A systemic (serum IgG and IgM) immune response is expected, as a high frequency of antigenaemia[57] and detectable viraemia[58] is observed in children with rotavirus gastroenteritis.

Natural immunity against severe disease has both serotype-specific and heterotypic components. There is evidence demonstrating that repeated infections with the same G serotype are less likely to occur[2], suggesting homotypic protection. However, this trend has not been observed in all studies and there is also clear evidence for heterotypic protection against severe disease after infection with a single serotype[2].

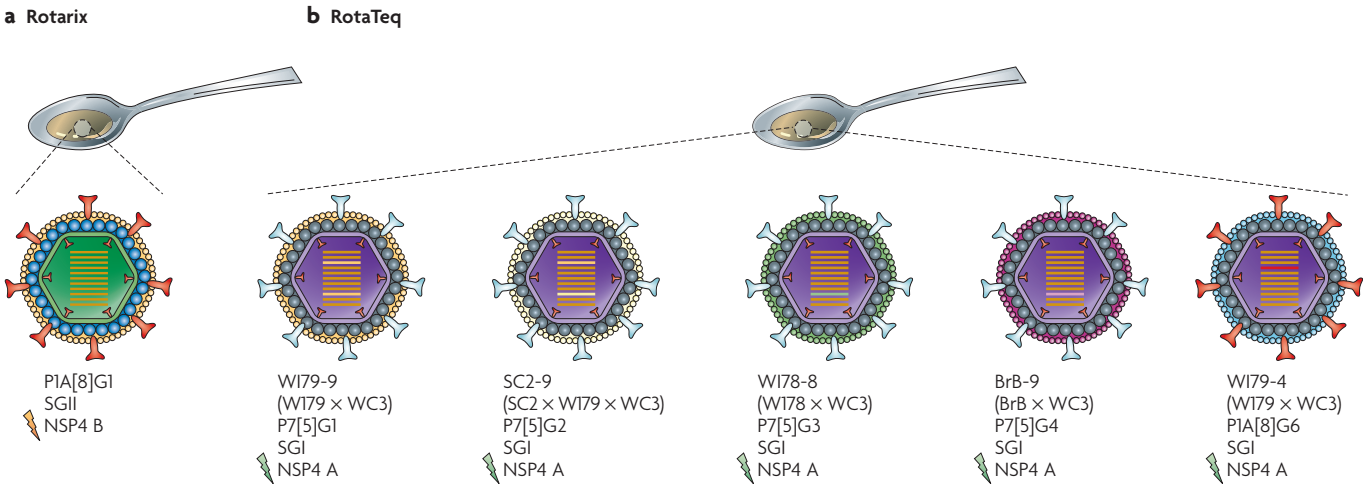

Figure 3 | **The Rotarix and Rotateq vaccines. a** | Rotarix is an attenuated human rotavirus vaccine made of a tissue-culture-adapted human P1A[8]G1, VP6 subgroup II and NSP4 geno-group B strain. **b** | RotaTeq is a bovine (WC3)–human reassortant vaccine composed of the five strains shown, each containing a human rotavirus gene encoding the VP7 neutralizing protein from different serotypes. Notably, in the WI79-9 and SC2-9 viruses (the last was used to create the first), genes 3 (VP3) and 9 (VP7) are of human origin. Although VP6 and NSP4 can potentially be the targets of protective antibodies (FIG. 2), their role in immunity against disease in humans is unknown.

includes two bovine rotavirus-neutralizing antigens (G6 and P7[5]) and five human rotavirus gene products. In the process of developing RotaTeq, the induction of serotype-specific neutralizing antibodies was considered crucial for protective immunity, and these antibodies were therefore the gold standard for measuring immunogenicity to the vaccine[8]. The WC3 bovine virus grows well *in vitro* (yielding high-titre virus for inoculation) but seems to replicate relatively poorly *in vivo* (the vaccine is excreted by less than 6% of children[59]).

RotaTeq immunity and efficacy studies. Selected studies that have evaluated the protective efficacy and immune response in infants who were vaccinated with individual components of RotaTeq or the complete pentavalent RotaTeq vaccine are summarized in TABLE 2. This table highlights several important observations. First, initial studies using a vaccine containing only the parental WC3 bovine strain showed variable levels of efficacy. These studies were generally done with only one[60,61] or two[62] doses of the vaccine and are therefore not directly comparable to studies of the WC3 reassortant vaccine candidates, which were mostly carried out using three doses of vaccine (TABLE 2). Consequently, there is suggestive, but not definitive, evidence that the reassortant vaccine strains that carry human rotavirus genes encoding the VP4 or VP7 neutralizing antigens provide better protection than the WC3 parental strain.

Second, vaccines that are composed of only a G1 mono-reassortant (Trials 3 and 4 in TABLE 2), or a different combination of some of the five final reassortants (Trials 5, 6 and 8) and the final pentavalent RotaTeq formulation itself (Trials 7 and 10), induced comparable levels of protective efficacy against any rotavirus gastroenteritis and severe rotavirus gastroenteritis. By contrast, a vaccine that was composed of only the P1A reassortant (Trial 9) induced lower levels of protection. Notably, in the majority of trials presented in TABLE 2, the predominant rotavirus strain circulating in the community at the time of evaluation was the G1 strain, and in only one trial (Trial 10), which included more than 34,000 children, could the protective efficacy of the vaccine against rotavirus of individual serotypes or genotypes be assessed. Taken together, these observations suggest that, as seen previously with RotaShield[2,63], the G1 reassortant alone might be sufficient to induce levels of protection against rotavirus gastroenteritis that are comparable to that provided by RotaTeq. Additionally, although RotaTeq can induce efficient protection against any gastroenteritis and severe gastroenteritis induced by G1, G2, G3, G4 and G9 rotavirus strains[6], it is unclear which of the components are necessary to induce protection against rotavirus of different serotypes.

Third, a detailed analysis of the correlates of protection that are induced by RotaTeq or any of its components has not been published. In spite of this, it is interesting to highlight that, in general, neutralizing antibodies against G1 appear to correlate with the levels of protection against any rotavirus gastroenteritis, whereas the levels of total anti-rotavirus serum IgA seem to be higher and closer to the levels of protection afforded against severe gastroenteritis (TABLE 2). Notably, the levels of neutralizing antibodies induced against the different components of RotaTeq vary considerably, whereas the levels of protection against different serotypes do not (TABLE 2). As a consequence, although the levels of neutralizing antibody against G1 generally correlate with protection against any rotavirus gastroenteritis, RotaTeq can provide protection against severe disease in the relative absence of these antibodies. The P1A reassortant vaccine induced a relatively good rotavirus-specific serum IgA response but was a poor inducer of neutralizing antibodies and protection. Thus, the serum IgA induced by this vaccine does not seem to be protective.

Early studies on the immunogenicity of the G1 reassortant showed that infants younger than 4 months of age developed poor neutralizing-antibody responses to a single dose of the vaccine[64]. Although most children acquired a response to WC3 (probably as a result of antibodies to VP4) after the first dose, a progressive increase in the response to G1 was observed with each subsequent dose[64–66], and maternal antibodies to G1 appeared to impede the development of G1 antibodies in the youngest children. In a trial that evaluated the stool IgA response against a quadrivalent formulation (Trial 6 in TABLE 2), the frequency of responses after doses 1, 2 and 3 were similar, indicating that each of the three doses of vaccine elicited a booster response[66]. Hence, for vaccine formulations containing one or more of these poorly replicating bovine-rotavirus-based strains, two or three doses seem to be necessary to achieve an optimal response, particularly for the induction of serum neutralizing antibodies to the human components. The ability of the multivalent RotaTeq vaccine to boost immunity on secondary or tertiary vaccination is one possible reason that it performed better than some monovalent reassortant formulations of the same vaccine, as monovalent vaccine formulations generally do not boost a response on subsequent vaccination.

Rotarix. The rotavirus that is present in Rotarix was derived from the 89-12 strain that was, in turn, isolated from a naturally infected child with rotavirus gastroenteritis[67]. Strain 89-12 was chosen because children who were infected with similar P1A[8]G1 strains developed broadly crossreactive neutralizing-antibody responses, and symptomatic or asymptomatic infection with these strains provided 100% protection against rotavirus gastroenteritis[67] in the following season. Hence, this vaccine was formulated to contain a single human rotavirus strain, on the basis of the assumption that one natural rotavirus infection in children can efficiently prevent a second severe infection[1]. An attenuated version of the 89-12 strain (passaged multiple times in cell culture) was initially shown to be both immunogenic[68] and protective in infants in the United States[69].

Rotarix immunity and efficacy studies. The clinical trials with this vaccine (summarized in TABLE 3) have been carried out primarily in Latin America and Finland, and are still ongoing in other parts of the world, including Asia and Africa[70]. Neutralizing antibodies have been measured in only three of these trials (Trials 2, 3 and 7 in TABLE 3) and the levels observed were significantly below the level of protection. Like the studies of the WC3-based vaccines discussed above, neutralizing antibody responses are clearly age restricted[71]. Importantly, children receiving the Rotarix vaccine (typically 8 weeks old) develop low levels of neutralizing antibodies[71] but are well protected (Trial 6 in TABLE 3). Investigators have also associated the lower capacity of younger children to make neutralizing antibodies with the higher pre-immune levels of these antibodies (probably of maternal trans-placental origin[71]), which might mask the detection of neutralizing-antibody seroconversion or specifically inhibit the response to the rotavirus vaccine[71]. The second possibility seems more probable, given the results of recent experiments in neonatal pigs that show maternal antibodies can inhibit the induction of rotavirus memory B cells[72,73].

Table 2 | **Selected WC3-based (RotaTeq precursor) vaccine studies**

Trial	Vaccine formulation		Percentage of children with:		Percentage protection		Comments	Refs
	Vaccine virus*	Number of doses‡	Neutralizing antibodies§	Serum IgA‖	Any¶	Severe#		
1	G6	1	G1: 9	49	NS	NS	103 vaccinees	61
2	G6	1	G1: 8	NR	76	100	49 vaccinees	60
3	G1	2	G1: 22	NR	100	100	38 vaccinees	103
4	G1	3	G1: 70–84	NR	64.1	87	197 vaccinees	65,104
5	G1/G2	3	G1: 67–73; G2: 26–35	85–90	73–87	NR	Protection NS owing to low amounts of rotavirus gastroenteritis	105
6	G1/G2/G3/G6/P1A	3	G1: 57; G2: 17	88	75	100	187 vaccinees	66,106
7	G1/G2/G3/G4/G6/P1A**	3	G1: 73–86; G2: ~16–22	93–99	68–74	68–81	237–276 vaccinees	59
8	G1/G2/G3/G4	3	G1: ~80; G2: ~17	98	74	78	201 vaccinees	59
9	P1A	3	P1A: ~24; G1: ~5; G2: ~1	69	43	53	268 vaccinees; protection NS against any rotavirus gastroenteritis	59
10	G1/G2/G3/G4/G6/P1A	3	G1: ~76; G2: ~35	95	74	98	2,207 vaccinees	6

*Serotype of rotavirus included in the vaccine (all vaccines included the P7 determinant of the WC3 virus and most polyvalent vaccines included the VP7 determinant of the WC3 virus). ‡The potency of each dose varies slightly between studies. §Percentage of children with neutralizing antibodies against selected rotavirus strains. ‖Percentage of children with rotavirus-specific IgA seroconversion directed primarily at the non-neutralizing VP6 protein. ¶Percentage of children that were protected against any rotavirus gastroenteritis; for trials 7–9 protection shown is against rotavirus gastroenteritis caused by viruses with serotypes present in RotaTeq. #Percentage protection against severe gastroenteritis (the definition of severe gastroenteritis varies between studies). **Children receiving a high- and a middle-potency dose of the vaccine are reported. NR, not reported; NS, not significant.

Rotavirus-specific serum IgA appears to be induced at similar frequencies to the induction of protective immunity against any rotavirus gastroenteritis, but at clearly lower levels than occur during protection against severe disease (TABLE 3). In addition, although children with rotavirus-specific serum IgA were in general better protected than children without IgA (Trials 3 and 5, but not Trial 8, in TABLE 3), some children with rotavirus-specific serum IgA were re-infected, and no titre of rotavirus-specific serum IgA was specifically correlated with protection. However, few children with vaccine-induced rotavirus-specific serum IgA responses developed severe rotavirus gastroenteritis[74,75]. Hence, although protection against severe rotavirus gastroenteritis can occur in the absence of rotavirus-specific serum IgA, the presence of this IgA appears to be a good indicator of protection against severe rotavirus gastroenteritis[74, 75].

As in the RotaTeq studies, in most of the Rotarix studies (TABLE 3) G1 viruses predominated in the community. In one Latin American trial[74] and the large trial in Latin America and Finland (Trials 5 and 6 in TABLE 3) it was possible to determine vaccine efficacy against rotavirus of individual genotypes. In this last trial, the Rotarix vaccine induced more than 85% protection against severe gastroenteritis caused by G1, G3, G4 and G9 rotavirus strains (most share the P1A[8] determinants of the vaccine) and non-significant protection against severe disease associated with G2 strains (most of which share only the neutralizing epitopes that are common to the P1A and P1B serotypes). However, a subsequent meta-analysis of more clinical trial data showed that the vaccine protected 81.0% (95% confidence interval: 31.6–95.8) of children against any gastroenteritis and 71.4% (95% confidence interval: 20.1–91.1) against severe gastroenteritis caused by P[4]G2 (REF. 7 and B. De Vos *et al.*, personal communication). It is worth noting that, in contrast to RotaTeq, boosts in antibody levels following a second dose of Rotarix are relatively rare, and the primary effect of the second dose is to provide a fill-in immunization (no increase in titres in sero-positive vaccinees, but an increase in the rate of response in the total vaccine population)[74,76] by capturing children that did not appear to be immunized after the first dose.

Hence, it is clear that, although we now have two safe and highly efficient rotavirus vaccines, their development has been highly empirical, leaving large gaps in our understanding of how they induce protection. One practical consequence of this is that there is no satisfactory correlate of protection following rotavirus vaccination[2]. At present, the only practical way to evaluate new rotavirus vaccines is by carrying out large and expensive clinical trials. Studies in mice[32,33] and pigs[77] indicate that localization of the rotavirus-antibody-secreting cells to the intestine is a crucial factor in determining protective efficacy[2] after natural infection or live-virus immunization. Unfortunately, to date, accurate surrogate circulatory markers of intestinal immune status have not been found[78].

Other vaccine issues that require further study. Although, as we have seen above, both licensed rotavirus vaccines have an excellent efficacy record, their capacity to prevent rotavirus mortality in the least developed countries of the world, particularly in Africa and Asia, is still unclear. In the past, oral rotavirus vaccines[79–81] and vaccines against other enteric diseases[82] have performed less efficiently in developing than in developed countries, and it is unknown how the two new rotavirus vaccines will work in different regions. Studies of RotaTeq and Rotarix that are either in place or scheduled to start shortly in both sub-Saharan Africa and Asia will be key to clarifying this issue. Multiple variables could be responsible for oral vaccines working less efficiently in developing countries. Some variables might allow the pathogen to challenge better. For polio virus, population density (which can lead to increased transmission)[83], and the fact that transmission is highly seasonal in temperate climates but occurs year-round in

Table 3 | **Selected 89-12-based (Rotarix precursor) vaccine studies**

Trial	Age of children*	Percentage of children with:		Percentage protection		Comments	Refs
		Neutralizing antibodies‡	Serum IgA§	Any‖	Severe¶		
1	6–26	35	95	NR	NR	US study; protection not evaluated; 21 vaccinees	68
2	10–16	69	92	89	100	US study; 108 vaccinees	69
3	8	NR	80	73	90	Finnish study, children with IgA better protected; 245 vaccinees	75
4	5–15	NR	78	NR	NR	US study; doses of $10^{5.2}$ and $10^{6.4}$ have comparable immunogenicity; 271 vaccinees	76
5	8	NR	61–65	70	86	Latin American study; children with IgA better protected; 464 vaccinees	74
6	8	NR	NR	NR	100	Latin American and Finnish study; 9,009 vaccinees	7
7	16 24	75 79	100 100	NR	NR	US study, protection not evaluated; 20 and 14 vaccinees, respectively	71
8	8	NR	54**	75	100	Latin American study, less immunogenic formulation of vaccine, children with IgA not better protected; 159 vaccinees	78

*Age of children in weeks at first dose. ‡Percentage of children with neutralizing antibodies against the vaccine rotavirus. §Percentage of children with rotavirus-specific IgA sero-conversion mainly directed at the non-neutralizing VP6 protein. ‖Percentage protection against any rotavirus gastroenteritis. ¶Percentage protection against severe gastroenteritis (the definition of severe gastroenteritis varied slightly between studies and with WC3 based vaccine studies in Table 2). **Only IgA and not sero-conversion was measured in this trial. NR, not reported.

Box 3 | **Status of other vaccines that have been tested in clinical trials**

To date, all rotavirus vaccines tested in children have been either live viral vaccines of a Jennerian or modified Jennerian nature or attenuated human rotaviruses[9]. The Jennerian RIT 4237 vaccine (consisting of a P6[1]G6 tissue-culture-adapted bovine strain) is one of the rotavirus vaccines that was found to be safe and efficacious in trials in a developed country (Finland), but not efficacious in developing countries in Africa. The Lanzhou lamb rotavirus (LLR) strain, P[10]G12, which was developed in China, has been in use in that country since 2000; however, controlled clinical trial data supporting its efficacy and safety have not been published in the English literature. A bovine (UK)–human reassortant modified Jennerian candidate vaccine has been developed by the US National Institutes of Health (NIH). This vaccine was tested in Finland and provided a protective efficacy of 60% against any rotavirus gastroenteritis and 90% against severe gastroenteritis[101]. In a new approach to vaccine development, the NIH has licensed this vaccine to seven companies in three developing countries, where it is currently under development and evaluation. Finally, developed on the basis of the observation that neonatal rotavirus infections are generally asymptomatic and can protect against subsequent severe rotavirus gastroenteritis, two rotavirus vaccines derived from human neonatal rotaviruses are under study. The Indian human neonatal strain, strain 116E, is an unusual naturally occurring P[11]G9 human–bovine reassortant strain that is shed efficiently in the stool of newborns. In preliminary studies, the 116E vaccine induced a good immune response after a single dose. Nevertheless, more extensive trials of this vaccine are needed, as natural infection with a related neonatal strain (P[11]G10) did not induce protection[102]. An Australian group has developed a vaccine candidate that is derived from the RV3 P[6]G3 neonatal rotavirus strain, but this candidate has not been highly immunogenic in preliminary studies[9].

tropical developing countries have been implicated as factors in vaccine failure[84]. Rotavirus also has the same contrasting temporal distribution between developed and developing countries and, additionally, higher infectious doses and/or co-infection with multiple strains seem to occur in developing countries[21,22]. Furthermore, the genotypes and/or serotypes of strains circulating in developing countries frequently differ from the common G1P[8] strains circulating in developed countries[21]. Other variables lower the immunogenicity of vaccines. Lower immunogenicity of other enteric vaccines (including vaccines against poliomyelitis, shigellosis and cholera) in less developed countries has been associated with bacterial overgrowth and/or helminth co-infections[82,83]. Higher levels of pre-immune (maternal) antibodies in children in developing countries has also been suggested as a factor that is involved in the reduced immunogenicity of rotavirus vaccines[79,81,85]. Although some of the clinical trials of Rotarix involved children in middle-income Latin American countries[7] and the vaccine has been shown to work equally well in children who were relatively malnourished compared with those who were not[86], there are still no efficacy data from controlled clinical trials carried out in developing countries in Africa or Asia.

Some potential safety issues for the two currently licensed live-viral vaccines also remain. The incidence of intussusception associated with the first dose of the RotaShield vaccine clearly increased with age; children older than three months of age accounted for 80% of cases of intussusception but received only 38% of the first doses[5,87]. The large Phase III clinical trials of RotaTeq and Rotarix showed that these vaccines have a different intussusception profile from RotaShield. However, in these trials, almost all of the first vaccine doses were administered to infants who were under 3 months of age[6,7] and it is unknown whether these vaccines might induce intussusception more frequently if the first dose was given to older children. For this reason, the American Academy of Pediatricians recommends that the first dose of RotaTeq should be administered between 6 and 12 weeks of age and that immunization should not be initiated for infants who are older than 12 weeks of age[88]. To date, post-marketing surveillance looking for an association between RotaTeq and intussusception has identified fewer cases of intussusception in vaccinees than expected. Nonetheless, the recently modified (after 1 year of the vaccine in the market) FDA-approved label for RotaTeq includes a new sentence stating that: "In post-marketing experience, cases of intussusception have been reported in temporal association with RotaTeq." Finally, it should be noted that Rotarix has been found to significantly prevent intussusception and the same trend has also been observed for RotaTeq (B. De Vos and M. Ciarlet, personal communication). Thus, rotavirus vaccines might prevent low levels of natural rotavirus-induced intussusception or, possibly, provide non-specific protection against infection with other agents that cause intussusception. Although the net beneficial effect of rotavirus vaccines on intussusception is encouraging, it is clear that more studies of intussusception in humans[89] and in animal models (rotavirus promotes lipopolysaccharide-induced intusssusception in mice) are warranted[90].

Concluding remarks

Following the withdrawal of RotaShield, the rotavirus vaccine field has recovered and gone on to make great progress with the development of the two recently licensed vaccines. Nonetheless, the development of any future rotavirus vaccine and improvement of the currently licensed vaccines is still hampered by our limited knowledge of the mechanisms of rotavirus pathogenesis and the basis for protection against rotavirus-associated gastroenteritis in humans[2]. Although more work in this area is clearly needed, the rotavirus vaccine pipeline is healthy (BOX 3). Early studies in mice and rabbits[91,92] showed that parenteral immunization is effective against rotavirus. As a follow-up, alternative strategies for administering rotavirus vaccines are being evaluated, including parenteral and mixed parenteral–mucosal immunizations in monkeys[93] and pigs[72] or intra-rectal vaccines in mice[94,95]. Presumably, these alternative strategies would avoid or further diminish the risk of intussusception. In humans, a straightforward proposal to diminish the risk of intussusception is the administration of the existing live oral vaccines to children in the neonatal period, when intussusception is rare[96]. Careful surveillance for the induction of EHBA should be undertaken when performing such studies in neonates. Strategies to develop non-replicating recombinant protein, DNA[97] and/or virus-like-particle-based[98] vaccines could also provide safe and effective alternatives. Long-range goals might include the development of chemically defined vaccines that are rationally designed with a detailed knowledge of the T-cell[99] and B-cell[100] epitopes that confer protection against rotavirus, combined with improved knowledge of how to stimulate mucosal immunity and lymphocyte trafficking to the intestine.

Parenteral
A vaccine administered by injection into the muscle, subcutaneous tissue or dermis (as opposed to mucosal immunization via an oral or nasal route).

Jennerian vaccines
Vaccines derived from microorganisms that infect animals and which are naturally attenuated in humans owing to host-range restriction.

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Competing interests statement
The authors declare competing financial interests: see web version for details.

FURTHER INFORMATION
Juana Angel and Manuel Franco's homepage:
http://www.javeriana.edu.co/Genetica/html/010103.htm
Access to this links box is available online.

Biographies

Harry Greenberg is professor of medicine, microbiology and immunology, and is senior associate dean for research at Stanford University School of Medicine, Stanford, USA. His current research focuses on pathogenic viruses that infect the gastrointestinal, liver and respiratory tract. He is particularly interested in molecular mechanisms of pathogenesis, viral determinants of protective immunity, the molecular basis of host-range restriction, virulence and tissue tropism, vaccine development and epidemiology, with specific emphasis on the role of enteric viruses in less developed countries. He helped develop the RotaShield vaccine, the first rotavirus vaccine to be marketed in the United States.

Juana Angel is a professor at the Instituto de Genética Humana of the Medical School of the Pontificia Universidad Javeriana in Bogotá, Colombia. She obtained her Ph.D. with Catherine Fournier (Paris, France) on a cellular biology model of rheumatoid arthritis, and undertook a postdoctoral fellowship with Harry Greenberg at Stanford University studying mechanisms of immunity to rotavirus in mice. She currently co-directs a research laboratory that is focused on the determinants of protective immunity induced by natural rotavirus infection and vaccination in children.

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TOC Blurb

Rotavirus is responsible for >500,000 deaths in children under 5 years of age worldwide annually. Two new vaccines shown to protect against rotavirus gastroenteritis were recently licensed. Angel, Franco and Greenberg review recent advances in our knowledge of the virus, the host immune response to rotavirus infection and the efficacy and safety of the new vaccines.

Online Summary

- Rotaviruses are the single most important aetiological agent of severe gastroenteritis in children. They are responsible for the death of approximately 1,600 children each day worldwide, mostly in developing countries.
- Two new rotavirus vaccines have recently been shown to be safe and effective in protecting young children against severe rotavirus gastroenteritis.
- These vaccines were designed using different approaches: the first (Rotarix) is an attenuated human rotavirus that is representative of the most frequently circulating rotaviruses. The second (RotaTeq) is composed of five rotavirus strains, which are all derived from a parental bovine rotavirus strain and contain a gene from rotaviruses of human origin.
- Ongoing clinical trials will be key in determining whether these two vaccines are efficacious in the poorest areas of the world, where they are most needed. As for other vaccines, post-marketing studies are ongoing to round up the efficacy and safety profile of the vaccines.
- Improvement of the two new vaccines and development of the next generation of rotavirus vaccines is hampered by our limited knowledge of the mechanisms of rotavirus pathogenesis and the basis for protection against rotavirus-associated gastroenteritis.
- Studies of the rotavirus mucosal immune response, and in general of the immune response of children, will be important for the development of correlates of protection for rotavirus vaccines.

Links

Juana Angel and Manuel Franco's homepage:
http://www.javeriana.edu.co/Genetica/html/010103.htm

Competing financial interests

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