Genetic and Antigenic Diversity of Human Rotaviruses: Potential Impact on Vaccination Programs

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The successful development and implementation of rotavirus vaccines will have a significant impact on rotavirus-induced gastroenteritis in children worldwide. However, this introduction will increase the immune pressure against wild-type rotavirus strains circulating in the community, altering the forces and balances that drive rotavirus evolution. There is concern that antigenically distinct novel or rare strains may be selected and spread, decreasing vaccine efficacy. This review describes the diversity of rotavirus strains and the mechanism by which wild-type rotavirus strains can evolve, including genetic drift and genetic reassortment.

Group A rotaviruses are the major cause of severe gastroenteritis in young children worldwide. Rotavirus strain and serological surveillance studies indicate that all young children are likely to have experienced ≥1 rotavirus infection by the time they are 5 years of age [1]. Rotavirus infection accounts for approximately one-third of all hospital admissions for diarrheal disease, and is estimated responsible for 400,000–600,000 deaths each year among children [1]. The majority of deaths occur in developing countries in Asia and sub-Saharan Africa, probably owing to delays in access to medical treatment. Although very few deaths occur in developed countries, good hygiene and sanitation do not appear to reduce the prevalence or prevent the spread of rotavirus infection. For example, 58,000–70,000 children are hospitalized annually in the United States with acute rotavirus diarrhea [2]. Although the symptoms of rotavirus diarrhea can be treated with rehydration therapy, prevention by vaccination is a better, long-term strategy. Two rotavirus vaccines have recently been developed and have proved safe and highly efficacious in large safety and efficacy trials [3, 4], leading to licensure in nearly 100 countries throughout the world.

Rotavirus, a genus of the Reoviridae family, contains a genome of 11 segments of double-stranded RNA coding 6 structural and 6 nonstructural proteins [5]. The RNA genome is located inside a triple-layered structure containing a core, an inner capsid, and an outer capsid [5]. The outer capsid is composed of 2 proteins, VP7 and VP4, both of which elicit neutralizing antibody responses that are serotype specific and serotype cross-reactive [6–8]. Given the segmented nature of the genome, the genes encoding VP7 and VP4 can segregate independently and thus are used in a binary classification system. Rotavirus serotyping is based on antigenic differences in the VP7 and VP4 proteins, which provides the basis for strain classification into G (VP7, glycoprotein) and P (VP4, protease sensitive) serotypes [5]. The classic serotype classification system has been replaced almost completely by a genotyping classification system based on sequence differences of the re-
spective gene segments. VP7 G serotype designation has been shown to largely agree with G genotypes; thus, a single nomenclature has been adopted. In contrast, a dual nomenclature has been adopted for the VP4 serotype and genotype classification. The P serotype, when known, is denoted by a Arabic number (eg, P1A), and the P genotype is denoted by an Arabic number within squared brackets (eg P[8]). If both are known, the P serotype precedes the P genotype (eg, P1A[8]). To date 16 G genotypes and 27 P genotypes have been identified, of which 11 G types and 12 P types have been recovered from humans [9, 10]. More recently, a genotype classification system for all 11 genome segments has been proposed for use in designation of new genotypes [11].

GENETIC DIVERSITY OF HUMAN ROTAVIRUS STRAINS

Worldwide, there is a great diversity in circulating wild-type strains. Dominant strains causing severe disease change from year to year and from country to country. This diversity of rotavirus is generated by several mechanisms. These mechanisms include (1) accumulation of point mutations (genetic drift) that can lead to antigenic changes; (2) reassortment that lead to viruses with novel genetic and antigenic characteristics, which can occur as a result of exchange between 2 human strains or human and animal strains; (3) direct transmission of animal strains into a human host; and (4) gene rearrangement (eg, deletions, duplications, and insertions) into coding or noncoding regions, primarily of nonstructural genes.

ROTAVIRUS PROTECTION

Primary rotavirus infection of young children does not confer immunity against reinfection, but it does protect against the development of clinically severe disease during rotavirus reinfection [12, 13]. In longitudinal studies, 88% of children were protected against severe diarrhea on reinfection after a single rotavirus infection [13]. This observation has become the strategic basis for administration of rotavirus oral vaccines. The first rotavirus infection elicits a predominantly serotype-specific (homotypic) neutralizing antibody response, and subsequent infections, even with the same rotavirus serotype, generally elicit a broader serotype cross-reactive (heterotypic) response [12–14].

The mechanisms of protection against clinical disease on reinfection are still unclear. However, it is generally accepted that mucosal and serum antibodies are associated with protection. The 2 outer capsid proteins, VP4 and VP7, play an important role by stimulating the production of neutralizing antibody [15, 16]. However, antibody response after rotavirus infection is broader and comprises antibody against various other rotavirus proteins, including VP2, VP6, NSP2, and NSP4 [17–19]. Increasing evidence suggests that viral proteins with-
In particular, G1P[8] strains have been consistently present and often predominant in most locations, representing >70% of strains in developed regions, such as the United States, the United Kingdom, and Australia, but often only 25%–50% in less developed regions, such as South America and Africa [9, 16]. G2, G3, or G4 strains generally represent minor causes of disease; however, outbreaks can occur at intervals of 2–5 years in many regions.

Rotavirus vaccines (developed since 1983) were aimed at providing specific protection against severe disease caused by the 5 common serotypes of rotavirus (G1, G2, G3, G4, and G9). However, during the past 10 years a wealth of literature has also documented the emergence of rare or unusual VP7 genotypes that have been reported to cause severe disease in children. Rotavirus surveillance programs in Australia [28, 29], Bangladesh [30], Brazil [31], Cameroon [32], Hungary [33], India [34], Malawi [35], Nigeria [36], Thailand [37], and the United States [38] have identified genotypes such as G5, G6, G8, G10, and G12 as causes of severe disease in children. Although these genotypes represent minor types globally, in some regions of India, Africa, and Brazil, they can be a significant problem. For example, genotype G5 in Brazil in 1996 [39] and genotype G8 in Malawi in 1997–1999 [35] were important locally, representing 57% and 34.8% of strains, respectively. Thus, the diversity of rotavirus genotypes may have significant implications for vaccine development and successful implementation, especially if strains that are not targeted by current vaccine candidates emerge as common types, either globally or regionally.

The likelihood that other uncommon genotypes may become important and significant causes of acute gastroenteritis on a regional or even global scale is a major dilemma faced by vaccine manufacturers. The most obvious example is the recent emergence of genotype G9. Strains with a G9 VP7 protein were originally reported as single isolates in the early to mid-1980s. However, since the mid-1990s, they have been recognized to have a global distribution and have been identified on all continents, and in >17 countries, including the United States, Japan, India, Bangladesh, France, Malawi, Nigeria, Australia, China, Thailand, South Africa, Paraguay, Ghana, Brazil, and the United Kingdom [40–47]. Generally, genotype G9 represents a minor component of the circulating rotavirus population, with prevalence rates from 1% to 10%. However, higher rates have been occasionally reported, for example, in Australia (74.7%), Bangladesh (39%), and Ghana (26.9%) [40, 43, 46]. In Australia, G9P[8] strains were first identified during Australia-wide surveillance in 1997. The prevalence of G9 then steadily increased, from a minor type in 1998 and 1999 to the second most prevalent serotype in Australia during the next 2 years (2000 and 2001), comprising 10% and 18.1% of strains, respectively [46]. It then became the dominant strain nationally in 2002 (40% of strains) and in 2003 (74.7%) [48, 49]. Both licensed vaccines, RotaTeq and Rotarix, have demonstrated protective efficacy against G9P[8] strains [3, 50, 51]. Protection in this instance is likely to have been achieved via the P[8] component, contained in both vaccines.

The ability of current vaccine candidates to protect against other newly emerging or rare VP7 genotypes, such as G12, cannot be predicted. Therefore, continued surveillance of rotavirus genotypes circulating before and after the introduction of a vaccine is vital to determine future efficacy and the need to update vaccine components, analogous to strategies for influenza vaccines.

INTRODUCTION OF ANIMAL ROTAVIRUS STRAINS INTO HUMAN CIRCULATION

Several rotavirus genotypes, believed to be of animal origin, have been detected in low numbers in children with acute gastroenteritis [9, 10, 52]. Genotypes G6, G8, and G10 are major bovine rotavirus genotypes and all have been sporadically identified in children in most regions of the world. However, G8 strains have been identified in large numbers in Africa [35, 53], whereas G10 strains are frequently identified in Indian children [54]. Porcine genotypes G5 and G11 have also been sporadically isolated from children with acute gastroenteritis. As with the bovine genotypes, porcine types occur sporadically in humans. The notable exception was the endemic persistence of genotype G5 in Brazil [39].

In addition to fluctuations in prevalence of “emerging serotypes,” the ability of rotaviruses to undergo genetic reassortment could lead to the production of atypical or novel strains, in which single or multiple genes are exchanged between human-animal strains or by interspecies transmission of animal strains to humans. This is particularly likely to occur in developing countries, where children often become infected with multiple strains at the same time. For example, in Brazil 11%–30% of children with diarrhea had mixed rotavirus infections [55]. Fundamental differences in rotavirus epidemiology between developed and developing countries make it important to evaluate vaccines in both types of regions. There are many reports of genetic reassortants causing severe disease in humans. These reports are reviewed elsewhere [56–58].

GENETIC EVOLUTION: IMPACT OF GENETIC CHANGE IN CIRCULATING VIRUS POPULATIONS

As the emergence of new genotypes represents an important problem for vaccines, another major issue of protection involves the potential for genetic shift at immunologically important regions within a single genotype and the potential impact these changes have on protection. Most longitudinal studies of rotavirus variation have focused on changes identified...
in the outer capsid protein VP7, the major neutralization antigen of the virus. Comparative analysis of the deduced amino acid sequences of the VP7 gene for different rotavirus G serotypes has revealed discrete divergent regions, and ≥4 of these—regions A (amino acids 87–101), B (145–152), C (208–221), and F (235–242)—contain cross-reactive and serotype-specific neutralization epitopes [5–7]. The mapping of neutralization escape mutants has shown that these regions represent the antigenic and neutralization regions of the virus. Within each of the major VP7 G serotypes (G1, G2, G3, and G4), antigenic subtypes or monotypes have been identified using serotype-specific neutralizing monoclonal antibodies [5–8]. In each instance a genetic basis for variation in the antigenic regions has been identified.

The impact of the intraserotypic diversity of circulating rotavirus strains on vaccine efficacy is yet to be elucidated. Several studies provide valuable information about the genetic diversity of the rotavirus VP7, which suggests that variability exists within G types. Palombo et al [59] suggest that within each of the major VP7 serotypes causing human infection (G1, G2, G3, and G4), genetically distinct strains emerge from a pool of related community strains to become predominant each year, rather than evolving directly from a previous dominant strain. Serotype G1 strains have been shown to possess 4 distinct genetic lineages worldwide [60]. The global distribution of these lineages indicated that some locations consist predominantly of a single lineage, whereas others support a number of cocirculating lineages [61]. Similar lineages have been identified for serotypes G2 and G4 [62]. Serotype G4 strains appear to be more divergent than the other G types in some regions [63]. Serotype G9 strains provide the best example of how genetic lineages can evolve. Sequence analysis of G9 VP7 has revealed several distinct lineages in circulation worldwide [64, 65]. One can be linked to the original historical G9 strain identified in the 1980s in the United States and Japan, and strains identified since 1996 are postulated to have arisen by accumulation of genetic changes. Several G9 VP7 lineages have been associated with differences in gene segments VP4, VP6, and NSP4 [58, 66].

The genetic diversity of lineages within rotavirus VP7 has been studied using strains that have caused significant disease outbreaks. Epidemiological studies have shown that serotype G2 is often associated with cyclic peaks [62, 66]. The recent emergence of serotype G2 in the United Kingdom coincided with substitutions in the antigenic A region of VP7. Iturrza-Gomara et al [66] postulated that an altered antigenicity in these strains resulted in immune-system evasion of cross-reactive antibodies. In South Africa, outbreaks of acute gastroenteritis due to serotype G2 infection occur every 3–4 years, with major epidemics occurring every 10 years [62]. Analysis of the antigenic regions of the VP7 gene of G2 strains isolated during a 15-year period (including 2 major epidemics) revealed 3 distinct patterns. Specific genetic changes in the antigenic A, C, or F regions were associated with each new appearance [62]. These altered epitopes in antigenically important regions of the VP7 could be implicated in epidemics of acute gastroenteritis.

There is limited information about the genetic and antigenic variation of the VP4 outer capsid protein. There appears to be a degree of polymorphism within the major P types, P[4], P[6], and P[8], with several lineages detected within each group [67, 68]. The relationship between genetic changes and antigenic differences has not been investigated.

Unfortunately, the impact of antigenic diversity and antigenic drift associated with emergence of dominant strains has been overlooked in many instances, possibly because of the development of molecular biology based methods, such as heminested multiplex reverse-transcription polymerase chain reaction assays. Studies designed to understand how genetic and antigenic changes in the VP7 and VP4 protein of strains may affect the successful implementation of rotavirus vaccines are still required.

CONCLUSION

There is a great diversity of wild-type rotavirus strains circulating worldwide, with dominant strains changing from year to year. Both rotavirus vaccines have been shown to protect well against the common strains causing disease, but protection against rare or novel strains is yet to be determined. As detailed in this review, in nature rotavirus is able to evolve rapidly, by accumulation of point mutations that can lead to antigenic change or by the emergence of new strains, possibly through reassortment between animal and human strains. It is not known whether this will occur more frequently with selective pressure after vaccine introduction. Novel or rare strains could emerge, with outer capsid combinations for which the vaccines do not protect. Thus, national and international epidemiological surveillance studies of the circulating strains, in terms of both genotype diversity and makeup, will be vital both to understanding future vaccine successes and to interpreting vaccine failures.

References

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