Molecular epidemiology of rotavirus in Brazil: A model for the tropics?

Abstract: The study of the molecular epidemiology of rotaviruses has recently moved from an early PAGE-based to a new PCR-based methodology, providing new information and greater meaning to the genomic diversity of rotavirus strains. Thus, the first studies involving characterization of both the G and P types of rotaviruses isolated in the United States and in Brazil have disclosed unsuspected features and major differences in the epidemiology of rotavirus genotypes. The contrasts observed in the two countries are most likely representative of those of a developed versus a developing country, or, of a temperate versus a tropical region. Continuous surveillance on major rotavirus types causing diarrhea in the tropics is essential for the formulation of an efficacious vaccine suitable for these regions of the world.

Introduction

Group A rotaviruses are important and widespread agents of gastroenteritis in the young of several animal species, including humans (19). In the developing world, they cause an estimated 17 million cases of moderate to severe diarrheal disease resulting in over 870,000 deaths per year in children

Key words: rotavirus epidemiology, G and P types, diversity, mixed infections, natural reassortants.
less than 5 years of age. In developed countries, similar morbidity rates are observed, and, although mortality rates are very low, rotaviruses are still responsible for a sizable economic burden. Therefore, vaccine development has been the subject of intense laboratory research and multiple field trials (2, 5, 19, 33). Rotavirus vaccine candidates have included several live animal rotaviruses (Jennerian approach), monoreassortants containing the VP7 gene of human serotypes G1 to G4 on an animal background (modified Jennerian approach), and human rotavirus strains obtained from asymptomatic neonates (nursery strains) which were thought to be naturally attenuated for children (2, 19).

**Molecular Epidemiology - from Electropherotypes to Genotypes**

As a member of the *Reoviridae* family, rotavirus is a double capsid virus with a segmented dsRNA genome (27). Both outer capsid proteins, VP7 (G type) and VP4 (P type), evoke antibodies that independently determine neutralization phenotype and greatly complicate the identification of an isolate G and P type by serological tests (19). Early inability to propagate human rotavirus isolates in cell cultures further contributed to the difficult task of producing serotype-specific reagents. Great emphasis was then placed on the virus genome, which became the focus of analysis of rotavirus field isolates during the late 1970’s and 1980’s (7). The 11 segments, or genes, of rotavirus can be easily separated by polyacrylamide gel electrophoresis (PAGE) producing migration patterns (electropherotypes) that are characteristic of each strain. The analytical power of the technique (the level of resolution of the genomic segments) is highly dependent on the PAGE conditions, thus precluding direct comparisons of electropherotypes among isolates analysed in distinct laboratories or even in distinct gels. Nevertheless, PAGE-based analysis produced most of what is known of the epidemiology of rotaviruses today. Some of the major findings included: (a) high diversity of strains, (b) cocirculation of several strains, (c) occurrence of mixed infections, (d) predominance of a strain in a community, (e) change of the predominant strain in a community, (f) appearance of new and disappearance of old strains from a community, (g) existence of many variants for each serotype.

Despite efforts to identify the antigenic characteristics by the electropherotype of the isolate, only limited and loose associations could be made between subgroup or serotype and electropherotype (7, 12). In the mid 1980’s, advances in molecular techniques and availability of sequence data on several rotavirus strains of distinct serotypes allowed the identification of specific regions for the design of type-specific primers for PCR assays and type-specific probes for hybridization assays (8, 11, 14, 15, 22, 23).
Table 1 - Correspondence among the various VP4 classification schemes for the P types discussed in this review.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>This review</th>
<th>Recommended</th>
<th>Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prototype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wa</td>
<td>P1</td>
<td>P[8]</td>
<td>P1A</td>
</tr>
<tr>
<td>DS1</td>
<td>P2</td>
<td>P[4]</td>
<td>P1B</td>
</tr>
<tr>
<td>M37</td>
<td>P3</td>
<td>P[6]*</td>
<td>P2A</td>
</tr>
<tr>
<td>Gottfried</td>
<td>P[shott]</td>
<td>P[6]*</td>
<td>P2B</td>
</tr>
<tr>
<td>HCR3</td>
<td>P6</td>
<td>P[3]*</td>
<td>P5A</td>
</tr>
<tr>
<td>RRV</td>
<td>P[RRV]</td>
<td>P[3]*</td>
<td>P5B</td>
</tr>
</tbody>
</table>

* No distinction of subtypes.

Those alternative techniques and, in particular, the PCR-typing assays for “human” and “animal” G and P types (8,11,14,15), have already generated new and exciting information which is the subject of the present review.

Nomenclature/Classification - Genotype x Serotype

A complete correlation between G genotype (determined by molecular methods) and G serotype (determined by neutralization methods) has been shown thus far (11,19). Therefore, G type refers to both genotype and serotype.

Several classifications have been reported for the VP4 specificities, or P types. In order to be consistent with most of the literature cited here, the original classification of Gorziglia et al, 1986 (10), Larralde & Flores, 1992 (22) and Gentsch et al, 1992 (8) for P genotypes that was extended by us (15,23) has been maintained. Correspondence with a recently recommended nomenclature for P genotypes and the present classification for P serotypes is shown in Table 1 for the types relevant in this review. For a complete listing see reference (19).

Of note, the recommended classification does not distinguish the subtypes (P2A from P2B; P5A from P5B) whereas the original classification shows well that the PCR-typing techniques (8,15,23) can clearly distinguish the subtypes. Therefore, the original classification fully correlates with the serotyping classification, though not with the nomenclature (Table 1).

Rotavirus G and P types: United States x Brazil

A comprehensive survey on the distribution of G and P types was conducted in the United States involving seven sentinel centers covering distant regions of the country (12, 29). It was the first survey to
demonstrate the utility of the recently developed PCR-typing method, and, to date, remains the only study to identify both G and P types of large numbers of rotavirus isolates in the United States. The location, period of study, and number of samples typed from each collaborating center is shown in Fig.1.

All rotavirus isolates were obtained from children with symptomatic infections during the rotavirus season that spans from October to April of each year. Stool specimens were collected from children admitted with gastroenteritis or who developed gastroenteritis during their stay in five Children’s Hospitals (Buffalo, Philadelphia, Denver, Washington, and San Diego), from children with diarrhea attending day-care centers in Houston, or were specimens sent to a large Atlanta-based diagnostic laboratory serving the southeast region of the country (Smith-Kline Research Laboratory).

Uneventfully, as shown in Fig.2, all isolates were of the common G1 to G4 types, with an overall prevalence of G1 strains, as has also been found in vaccine efficacy trials and other studies in the north and northeast United States (5,24,33). For the VP4 specificity, as expected, P1 was overwhelmingly prevalent (Fig.3) and associated with types G1, G3 and G4, whereas P2 was exclusively associated with G2 (29). Only a few samples contained mixtures of distinct G or P types, and no sample was left untyped. Surprisingly, three isolates (one G2 and two G1) bearing P3 were found (30). This specificity had not previously been reported in the United States. The G1P3 isolates were recovered from nosocomially infected infants suggesting that nursery strains might also be common in this country, and might occasionally cause severe disease (see “nursery strains” below).

A very different scenario was observed in Brazil. A survey on the distribution of rotavirus types conducted in the state of São Paulo demonstrated that only half of the isolates were of the common G1-G4 types (Fig.4) and 29% were of the common P1-
P2 types (Fig.5) (31). No single type, including P1, was overwhelmingly prevalent, but rather they were all well represented. Remarkable, however, was the discovery of some interesting features in extraordinarily high proportions of the isolates, a finding that had not previously been described anywhere. Those features were:

**G5 - A new epidemiologically important G type**

Rotavirus type G5 was first recognized as a swine pathogen in the United States (prototype strain OSU) and later found in many other countries including Australia, Venezuela and Argentina (3). A single isolate (strain H1), very similar to OSU was recovered from a foal in Great Britain, but the virus appears not to be prevalent in horses.

In humans, G5 rotaviruses were first found in children with gastroenteritis in Brazil (16). Rather than being a rare event, G5 strains were found widely dispersed in the country for extended periods of time (Fig.6). Some isolates were from an outbreak of gastroenteritis in Rio de Janeiro that affected several adults; others were from an outbreak in a day-care center in São Paulo that produced secondary familial cases of gastroenteritis, indicating person to person transmission. Furthermore, G5 was the prevalent serotype in the state of São Paulo in 1992, and, together with G1, the second most prevalent during the eight-year study period (Fig.4) (31). Those observations clearly demonstrate the epidemiological importance of type G5 in Brazil, and most likely in other South American countries as well (18).

Antigenic characterization of one of the G5 strains, IAL-28, revealed an unexpected and novel association between the human strain and yet another “porcine serotype”, G11 (32). The G11 serotype was originally described as prevalent and widespread among pigs in Mexico (prototype strain YM), and later found in pigs in Venezuela and in calves in the United States (4,21,28). The VP7 of IAL-28 was shown to contain antigenic determinants specific for both G5 and G11 serotypes (32). This was the first
human and second rotavirus strain, after the G3/5 porcine strain MDRC13, found to express dual VP7 specificity (25). Studies are underway to examine whether the other G5 isolates also possess the dual G5/11 specificity.

**P3 - “Nursery strains” causing diarrhea in many children**

Strains bearing all four major G types have been recovered from healthy neonates in hospital nurseries in Australia, Sweden, Venezuela, England, and, most recently in France (20, 30). Those “nursery strains”, as they became known, were endemic in some hospitals for years, asymptptomatically infecting neonates. They were found to share the same VP4 specificity (P3), that was quite distinct from those of common virulent strains (30). This observation led to the suggestion that gene 4 might be a genetic marker for rotavirus attenuation, and nursery strains might make safe and effective vaccine strains (2,10,20).

In the Brazilian study (31), a large proportion (11%) of the isolates recovered from children with moderate to severe gastroenteritis carried the P3 genotype (Fig.5). Rare, sporadic cases of diarrhea had been previously described in association with P3 rotaviruses in the United States (see above), Italy, Peru, Venezuela and Bangladesh (30). However, the high proportion of P3-associated diarrheal cases identified in non-neonates in Brazil seem to suggest that although attenuated for newborns, those strains might still be virulent in infants and older children.

**P6 - Unusual P type (canine-feline) associated with diarrhea**

An “animal-like” P type was suspected for a rotavirus isolate (HCR3) recovered from a healthy child in Philadelphia: it readily replicated to high titer in cell culture and presented unique restriction pattern of its VP7 copied cDNA (13). Sequence analysis of its gene 4 revealed a new P type that was more similar to some animal P types than to conventional human types (23). PCR typing of feline (Cat97) and canine (K9) strains revealed that those animal strains shared the HCR3 P6 type (15). A similar strain was identified in an Israeli child with diarrhea (R01845) (26).
Both HCR3 and Ro1845 were latter shown to share antigenic specificity with the canine and feline strains, and the human infections were attributed to rare instances of interspecies rotavirus transmission.

However, a high proportion (11%) (Fig.5) of P6 isolates was found in the Brazilian study, indicating that human infections with P6 rotaviruses are rather common events of possible epidemiological dimension. Future surveys on rotavirus types in Brazil and abroad including P6-specific reagents should clarify the distribution and importance of this new P type in childhood diarrhea.

G? P? - Other “animal” types? Unknown types?

Most studies aimed at typing rotavirus isolates have found some proportion (varying from 0% to over 50%) of non typeable samples. Lack of reaction with neutralizing monoclonal antibodies (MAbs) has been attributed to loss of the virus outer capsid or variations in epitopes (monotypes). The use of molecular typing methods have largely overcome these problems (12). Furthermore, in most studies, MAbs were only available for the common human types, placing isolates carrying minor “human” types, common “animal” types, unusual types, and unknown types in the category “untypable”. It is interesting to note that, in general, the proportion of untypables has been small among isolates recovered from developed countries and large among those recovered from developing ones (1,6,9,12,24).

Initially, 39% of the isolates from São Paulo were considered untypable with the “human pool” for G types (31). Later, about half of them were identified as G5 by the PCR typing assay using the “animal pool” of primers (16). Still good proportions of the remaining untypable isolates (21% G? and 20% P?) were later identified as bearing known G and P types (unpublished results). Most likely, the majority of the untypable isolates reported in other studies would similarly be identified by the PCR typing assays for human and animal types (8,12,14,15). It can be predicted that, in addition to G5, other major “animal” G and P types will be found in epidemiological proportions in human isolates obtained from children with diarrhea, particularly in the developing world (18).

Multiple types - Mixed Infections and Natural Reassortants

Studies based on type-specific MAbs in ELISA formats or on probe-hybridization typing techniques presented another limitation: the reaction of an isolate with MAbs of more than one specificity, or with more than a single probe, was regarded as non-specific reactions and usually excluded from the study. Thus, many studies probably have underscored the prevalence of even common types when in a mixture
of distinct virus types.

The PCR typing technique has both high sensitivity and analytical power, being able to detect and identify multiple templates in a single sample (17). Furthermore, confirmation of the typing product can easily be performed by restriction endonuclease analysis or, more costly and laboriously, by hybridization with labelled probes or sequencing. Therefore, even complex mixtures of G and P types can be usually resolved.

In fact, in the Brazilian study, an unforeseen diversity of genotypes could be demonstrated in a single specimen (Fig.4 and Fig.5). Almost a third (29%) of the specimens analysed from São Paulo contained multiple G and/or P types, roughly ten-fold higher than the proportion found in the American study (or anywhere else in the world)(12,31). Complex mixtures of two G types and three P types in the same specimen were documented, demonstrating an enormous potential for reassortment during mixed infections (18).

Concluding Remarks

The application of the PCR-typing methodology to the study of rotavirus isolates demonstrated several remarkable epidemiological features in Brazil, which were significantly distinct from those found in United States. Those findings may, at least in part, explain repeated failures in South America and Africa of vaccine candidates that had shown good efficacy when tested in the United States and Europe (2,19). Therefore, it is rather tempting to speculate that the extensive diversity and complexity of rotavirus isolates observed in Brazil may represent a model for other tropical countries.

Acknowledgements:

NS received support from FUJB and FINEP

REFERENCES


