Molecular Epidemiology of Rotavirus in Black Infants in South Africa

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The initial difficulties of cultivating human rotavirus in vitro and the antigenic relatedness of the group A rotaviruses have led many researchers to seek alternative approaches for the study of rotavirus disease in humans (10). Biochemical methods have become an important tool in attempts to characterize rotaviruses. The rotavirus genome consists of 11 discrete segments of double-stranded RNA which can be separated into distinct bands by electrophoresis. Gel electrophoresis of the genomic segments yields a recognizable pattern which is both constant and characteristic for a particular isolate (7, 10).

Most molecular epidemiology studies have analyzed rotaviruses by the electrophoretic profile produced by polyacrylamide gel electrophoresis (PAGE). Rotavirus outbreaks in a wide variety of geographical and environmental backgrounds have been studied (5, 12, 22, 27, 28). Characteristically in these studies, a single strain of rotavirus was predominant at any specific time, with less common types also occurring. Some studies have reported that certain strains of rotavirus as identified by the electrophoretype may persist for 12 months or more (9, 22).

In contrast to the extensive variation of rotavirus strains found in older children and the relatively short persistence of these strains in the community, the strains found in neonates are less diverse and persist in maternity units for longer times (13, 21, 22).

Recently, rotaviruses with the rotavirus morphology but without the common group antigen have been isolated from humans (4, 8, 18). The more commonly occurring group A rotaviruses besides sharing a common (group A) antigen also share a standard RNA electrophoretype (4, 20). The different rotavirus groups each have a unique RNA electrophoretype pattern (4) and can thus be identified by the RNA profile.

In this study the occurrence and seasonality of different rotavirus electrophoretotypes in black infants in Southern Africa were investigated.

MATERIALS AND METHODS

Specimens. Stool specimens were obtained from March 1983 to December 1986 from 1,571 black infants admitted to the gastroenteritis unit at Ga-Rankuwa Hospital (30 km northwest of Pretoria). The presence of rotavirus antigen was diagnosed by Rotazyme (Abbott Laboratories, Diagnostics Div.) enzyme-linked immunosorbent assay as reported previously (26). The rotavirus-positive specimens were subjected to PAGE.

Specimens were collected between May and August 1984 (150 specimens) and between May and August 1986 (173 specimens) from the neonatal unit at the hospital. This unit admits both term and preterm babies from the labor wards at the hospital, as well as from outlying clinics and home deliveries. These stool specimens were examined by electron microscopy for the presence of virus, and all rotavirus-positive specimens were analyzed by PAGE.

PAGE was also performed on 546 rotavirus-negative specimens, from both children and adults admitted to the hospital in 1986 with diarrhea, to screen for the presence of atypical rotaviruses.

RNA extraction. The crude fecal extracts were centrifuged to sediment any macroscopic debris. The clarified stool suspensions were mixed with 1/10 volume of 1 M sodium acetate containing 1% sodium dodecyl sulfate and incubated for 15 min at 37°C. Viral RNA was extracted by deproteinization with 1 volume of a 1:1 phenol-chloroform mixture at 56°C for 15 min and centrifugation at 10,000 × g for 3 min. The RNA was precipitated from the aqueous phase with 1/10 volume of 3 M sodium acetate and 3 volumes of ethanol at −20°C. The RNA was pelleted by centrifugation at 10,000 × g for 10 min, and the pellet was dried and suspended in 40 μl of sample buffer (0.12 M Tris hydrochloride, 0.1% sodium dodecyl sulfate, 15% glycerol, 0.001% bromophenol blue).

PAGE. Electrophoresis of the extracted RNA was carried out in 10% polyacrylamide slab gels with a 3% stacking gel, using the discontinuous buffer system described by Laemmli (16) without sodium dodecyl sulfate. Approximately 30 μl of each sample was loaded, and electrophoresis was performed at 100 V for 16 to 18 h at room temperature.

Silver staining. The gels were stained by using a modification of the method described by Herring et al. (14). The gel was fixed in 40% ethanol containing 10% acetic acid for 40 min and then in 10% ethanol with 0.5% acetic acid for a further 40 min. The gel was then soaked in an 11 mM silver nitrate solution for 40 min before being washed in distilled water. Finally, the gel was reduced in a solution of 0.75 M sodium hydroxide containing 0.3% formaldehyde. The reaction was stopped with 5% acetic acid.

Electron microscopy. Stool suspensions (10 to 20%) were made in distilled water. A 10-ml portion of the suspension was centrifuged at 1,000 × g for 10 min to sediment any...
macrophagic debris. The supernatant was centrifuged at 25,000 \times g for 15 min to sediment any bacteria present in the stool, and the resulting supernatant was ultracentrifuged at 30,000 \times g for 1 h at 4°C. The viral pellet was then suspended in a few drops of distilled water and mixed with an equal volume of 3% phosphotungstic acid (pH 6.5). A drop of the mixture was placed on a carbon-Formvar-coated 300-mesh copper grid. Any excess fluid was blotted off, and the grid was allowed to air dry before examination at 80 kV and a magnification of \times 40,000.

RESULTS

An RNA electrophoretic profile was observed with 70% of the stool samples analyzed (269 of 389). Both major classes of rotavirus electrophoretotypes were observed (i.e., the long and short RNA profiles), although differences existed between them. The short RNA profiles (i) occurred less frequently than did the longer electrophoretotypes (12% [33 of 269] and 88% [236 of 269], respectively), (ii) showed less variability than did the longer profiles, with only 2 electrophoretotypes seen compared to 12 different long RNA electrophoretotypes, and (iii) showed greater stability over time (whereas the long RNA profiles changed every 12 to 24 months, the shorter patterns remained in the community for longer periods [Fig. 1]).

Each year a number of different rotavirus strains, as defined by PAGE RNA electrophoretotypes, circulated in the community, with one strain exhibiting numerical predominance. There was a constant change in the predominant strain, with a new strain emerging as the most numerous each year (Fig. 1). Over time there was a constant change in the numbers and types of RNA electrophoretotypes, so that some strains ceased to exist (e.g., LA in 1984), whereas new strains emerged within the community (e.g., LL in 1986). Most of the strains existed for short periods of time; some, such as LG in 1984-1985, emerged and disappeared within the space of a few months. Most, however, persisted in the area for approximately 2 years before disappearing (e.g., LB and LF). The predominant RNA electrophoretotypes found each year are shown in Fig. 2.

Among the 323 babies sampled from the neonatal unit, 24% (78 of 323) were excreting rotavirus. PAGE was conducted on all the rotavirus-positive specimens, and RNA profiles were obtained with 71% (55 of 78), revealing the presence of a single RNA profile in 1984 and a single profile in 1986. Coelectrophoresis showed that the 1984 and 1986 RNA profiles were identical. The neonatal strain was shown to correspond to the strain designated LD, which was circulating in the community in 1984.

Among the 546 stool specimens screened for the presence of atypical rotaviruses, no atypical RNA electrophoretotypes were observed.

DISCUSSION

Between March 1983 and December 1986, a succession of 14 different human rotavirus electrophoretotypes were observed for the infants and young children admitted to Ga-Rankuwa Hospital with acute gastroenteritis. During each seasonal peak of rotavirus activity, several rotavirus strains (indicated by RNA electrophoresis) existed, with a predominant strain and less common types present. The succession of rotavirus strains occurred in a manner in which some
strains disappeared as new strains emerged. It was also found that, whereas some strains existed for very short periods, others persisted for up to 2 years. These results confirm the findings of other studies conducted with limited numbers of specimens, usually over limited time periods (9, 12, 22, 28).

A question has been raised as to whether the predominant strain of one season occurs as a "herald wave" during the previous season (10). The results of the present study are a little ambiguous. Certainly, strain LB, which was predominant during 1984, was present in small numbers during the winter of 1983 and circulated over the summer months at a low level. Similarly, strain LF, which was predominant during 1985, was first detected in a single patient in August 1984. These results indicate that the predominant strain does occur in low levels during the previous season. However, it is also apparent that new strains may suddenly emerge as the predominant strain; strain LL erupted into prominence in May 1986 with no previous occurrence having been observed.

Some studies, dealing with variants of the long RNA profiles, have suggested that there is a major shift in the prevalent electrophoretype every 2 to 3 years (22, 23). It is still unclear whether this genomic diversity would reflect a concurrent antigenic change. Other studies have reported the temporal appearance of short versus long electrophoretypes (25, 29). Because of the presumptive association of the short electrophoretype and serotype 2 rotaviruses, Uhnoo and Svensson (29) have suggested that this shift in electrophoretype prevalence indicates an antigenic change and may reflect a change in the immunity of the population at risk.

In our study, the short electrophoretype (strain SB) first appeared in May 1984, when it assumed epidemic proportions. After this initial outbreak, the SB strain was still present but at reduced levels. The change in the prevalence of the short pattern in 1984 and the sudden emergence of strain LL during 1986 may be indicative of a shift in serotype, with a consequent upsurge of activity in the community. However, it should be noted that the electrophoretype alone cannot be used to identify the serotype specificity of the virus isolate, because it has been shown that viruses which belong to the same serotype can have different electrophoretypes and vice versa (1).

Rotavirus has been shown to be endemic in the neonatal unit at Ga-Rankuwa Hospital, as it has in many maternity units worldwide (6, 21, 24). The occurrence of this virus in the unit over a long period (unpublished data) and the natural self-limited nature of the infection indicate that the virus is spread within the ward. The occurrence of a single RNA electrophoretype in the unit in 1984 and 1986 further demonstrates that a single strain of rotavirus is endemic in the ward. The infection occurs very early in life, being found in babies just 1 day old, which has been reported elsewhere (17) and also indicates nosocomial spread of the virus.

It has been recently reported that gene segment 4, whose product is possibly associated with viral virulence (19), may be shared by different nursery isolates (11). This raises the interesting possibility that the neonatal strains are biologically different from the virulent strains that occur in older children.

The question of whether an asymptomatic (or mild) neonatal infection confers protection against a later rotavirus infection associated with illness is important in terms of vaccine development and administration. Bishop et al. (3) have shown in a 3-year longitudinal study that neonatal rotavirus infection did not restrict reinfection but that it did confer significant resistance to disease severity during reinfection. The observations that (i) rotavirus infection can occur at a very early age (even in 1-day-old babies), (ii) the infection in neonates is usually asymptomatic, and (iii) neonatal infection may confer protection against severe rotavirus gastroenteritis provide important clues as to the possibility of administering a vaccine to newborn children, now that vaccine trials are under way.

In the search for atypical rotavirus RNA profiles, none were identified. The occurrence of atypical rotaviruses in countries other than China is very low (8, 15, 20), and it is possible that there are none in this region or that they occur at such low levels that they have not been detected.

In this study, a small portion of the samples tested each year (29 of 389), although giving negative results in different enzyme immunosassays (EIA), yielded an RNA profile with the group A pattern on polyacrylamide gels. These specimens may have contained viruses whose antigen is masked by antibody and is thus undetectable by EIA. Another possibility, however, is that these viruses do not possess the group A antigen. Rotaviruses with the group A antigen were shown to exhibit unusual RNA profiles (2), and these isolates may represent viruses without the group A antigen but with the group A RNA electrophoretype. Of the 29 rotavirus isolates which did not react in EIA, 12 were detected in 1986 alone and may represent an epidemic of these strains. It should be noted that these non-EIA-reactive strains did not
have a distinct electrophoretotype but had 4 of the 14 RNA profiles observed in the study period.

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LITERATURE CITED

