Necrotizing Enterocolitis and Cytomegalovirus Infection in a Premature Infant

abstract

Necrotizing enterocolitis is the most common gastrointestinal emergency in neonates. The etiology is considered multifactorial. Risk factors include prematurity, enteral feeding, hypoxia, and bacterial colonization. The etiologic role of viruses is unclear. We present a case of necrotizing enterocolitis associated with cytomegalovirus and Proteobacteria in a 48-day-old, ex-premature infant and discuss the effects of potential viral-bacterial interactions on host susceptibility to this disease. Pediatrics 2013;131:e318–e322

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KEY WORDS
premature infants, cytomegalovirus, necrotizing enterocolitis

ABBREVIATIONS
CMV—cytomegalovirus
DOL—day of life
NEC—necrotizing enterocolitis
PCR—polymerase chain reaction

Dr Tran conceptualized and designed the study, obtained and maintained institutional review board approval, performed the clinical data collection at the primary and referral hospitals, coordinated the gene-sequence data collection, maintained the study database, drafted the initial article, and revised and approved the final article as submitted; Dr Ferris reviewed and revised design of the study, performed advanced analyses on the bacterial gene sequences, provided specialized expertise in the interpretation of bacterial community data, reviewed and revised the article, and approved the final article as submitted; Ms Norori conducted the initial sample preparations, coordinated data collection, and critically reviewed and approved the final article as submitted; Dr Stark performed the postmortem examination, suggested and performed additional histology and immunochemistry tests, provided specialized expertise in the interpretation of pathology findings, and critically reviewed and approved the final article as submitted; Dr Craver served as mentor for Dr Stark, provided specialized expertise in the interpretation of histology and immunochemistry findings, and critically reviewed and approved the final article as submitted; Dr Dowd conducted the 454 pyrosequencing, provided initial analyses of the bacterial gene sequences, and approved the final article as submitted; and Dr Penn served as research mentor for Dr Tran, reviewed and revised design of the study, performed analysis of the data, reviewed and revised the article, and approved the final article as submitted.


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Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency in the neonatal period, with an incidence of 1% to 8% of infants admitted to NICUs and a mortality rate as high as 50%. The most consistent risk factors are prematurity, enteral feeding, hypoxia, and bacterial colonization. Aerobic and anaerobic bacteria and viruses have been isolated from infants with NEC, but no single etiologic agent has been identified. Although cytomegalovirus (CMV) infection has been detected in neonates after complications of suspected NEC, there have been only a few cases of confirmed NEC with concurrent CMV.

Little is known about the interaction between viruses and bacteria in human intestinal disease. The potentiation of intestinal disease by Norovirus and enhanced bacterial translocation of bacteria across the intestinal mucosal barrier in HIV infections suggest a possible pathologic relationship. We present a case of NEC in a 48-day-old, ex-premature infant with systemic CMV infection. The concomitant demonstration of CMV and Proteobacteria in the affected intestine suggests a potential viral-bacterial interaction.

CASE REPORT

Our patient was an African American male infant born at 25 1/7 weeks of gestational age, with a birth weight of 653 g. Pregnancy was complicated by prolonged rupture of membranes for 96 hours before spontaneous vaginal delivery. The infant was intubated in the delivery room, and Apgar scores were 8 and 8 at 1 and 5 minutes, respectively. After 3 days of mechanical ventilatory support, he was extubated to a high-flow nasal cannula. Trophic gavage feedings with expressed breast milk were started on day of life (DOL) 7 but discontinued soon after owing to bilious residuals. They were restarted on DOL 13, gradually increased, and changed to orojejunal feedings on DOL 19 because of feeding intolerance. After full continuous feedings were achieved on DOL 23, caloric density was increased gradually to 24 kcal per ounce. Premature adapted formula (Similac Special Care, Abbott Nutrition, Columbus, OH) was used when maternal milk was no longer available. Multiple courses of antibiotics were given for clinically suspected sepsis. Prolonged rupture of membranes prompted treatment with ampicillin and gentamicin for the first 7 DOL. Complete blood cell counts and platelet counts were within normal limits. He was subsequently treated with vancomycin and ceftazidime because of feeding intolerance, bandemia, and thrombocytopenia. He was again treated with vancomycin and ceftazidime on DOL 34 owing to abdominal distension and bandemia. All blood cultures were negative for aerobic and anaerobic bacterial infection. He required multiple transfusions of packed red blood cells and 1 transfusion with platelets. All blood products were tested and negative for CMV. On DOL 48, he developed fulminant NEC with radiologic evidence of extensive pneumatosis intestinalis and subsequently died, despite aggressive medical management.

METHODS

Postmortem examination was performed within 24 hours of death by using routine gross and histologic techniques. The finding of characteristic inclusion bodies prompted additional immunohistochemistry staining (diagnostic kit: CMV [DDG9/CCH2]; Cell Marque, Rocklyn, CA) of selected tissues, testing for CMV. Serendipitously, the patient was enrolled in an ongoing investigation of fecal microbiota in premature infants. This study was approved by the Louisiana State University Health Sciences Center institutional review board. After informed parental consent was obtained, serial weekly stool samples were collected for bacterial gene-sequence analysis. Additional fecal samples and an ileal mucosal scraping were obtained when the autopsy was performed.

Pyrosequencing analysis of polymerase chain reaction (PCR)–amplified 16S ribosomal RNA gene sequences was used to identify bacteria in clinical specimens. DNA was extracted from specimens by using a commercial DNA kit (QIAamp DNA micro kit, Qiagen, Valencia, CA). PCR amplification of the V3-V6 region of the 16S ribosomal RNA gene was performed by using primers (forward: 28F 5’GAGTTTGATCCTGCTG; reverse: 519r 5’GWTTAACGGCCGCGCCTG). Pyrosequencing was performed by the Research and Testing Laboratory (Lubbock, TX), as previously described.

The average number of sequences per sample was 5815 (range: 4377–7067). The average sequence length was 416 base pairs (range: 250–546 base pairs). Bacterial taxa were identified by using the Ribosomal Database Project Classifier (http://rdp.cme.msu.edu/). The Shannon-Weaver Diversity Index and relative abundance of taxa in each specimen were determined. Isolated DNA from 4 stool samples were sent to an outside laboratory for measurement by CMV-PCR (Viracor-IBT, Lee’s Summit, MO).

RESULTS

Autopsy findings included diffuse pneumatosis intestinalis and hemorrhagic foci involving both the small and large intestines. Gram staining of intestinal tissue revealed focal bacterial growth associated with subserosal blebs. Histologic examination and immunohistochemistry staining revealed characteristic CMV inclusions in lung, spleen, and intestinal tract (Fig 1).

Serial fecal samples revealed a fall in bacterial diversity and a shift toward...
Proteobacteria predominance before development of NEC. The last sample (collected 2 days before death, when the infant appeared well, tolerated full enteral feedings, and was not receiving antibiotics) revealed a radically decreased bacterial diversity and overwhelming predominance of the phylum Proteobacteria and family Enterobacteriaceae (primarily Klebsiella and unclassified Enterobacteriaceae). The same microfloral pattern was found in the postmortem ileal fecal and mucosal scraping samples (Table 1). Qualitative CMV-PCR demonstrated CMV DNA in 3 fecal samples obtained on DOL 10, 12, and 25 (Table 1).

DISCUSSION

There is strong evidence supporting bacterial colonization as a major risk factor for NEC. No cases of NEC have been described in the sterile fetal intestine.8 Pneumatosis intestinalis, a cardinal feature of NEC, is characterized by bacterial production of hydrogen gas in the intestinal wall.1 The infant described here had radiologic and autopsy documentation of extensive pneumatosis intestinalis. Focal bacterial growth was associated with the subserosal blebs. The ileal mucosa scraped from an area of pneumatosis intestinalis revealed an overwhelming predominance of Proteobacteria, a phylum implicated in the pathogenesis of NEC.10,11

The infant also had systemic CMV diagnosed at autopsy. CMV, a double-stranded DNA virus, is a member of the herpes family. Transmitted through contact with bodily fluids and secretions, including breast milk, vaginal fluids, and blood, its prevalence in the United States is estimated to be between 35% to 80%.12 The origin and time of onset of our patient’s CMV infection are unclear. No stigmata of congenital CMV were observed. Initial platelet counts, liver function tests, and bilirubin concentrations were normal; however, only 10% to 15% of congenitally infected infants show clinical signs at birth.12 Vaginal delivery after prolonged rupture of membranes could have exposed the infant to potentially infective vaginal secretions, but no symptoms of perinatally acquired CMV (eg, pneumonia, hepatitis, or encephalitis)12 were observed. CMV DNA was detected in the first available fecal sample; however, this sample was collected 2 days after the initiation of trophic breast milk feedings. CMV has been detected in maternal milk as early as 1 to 3 days postpartum.13,14 Yasuda et al15 reported that CMV was readily transmitted in breast milk but found that resulting infections were usually asymptomatic. Unfortunately, by the time of diagnosis, no prefeeding blood, urine, or breast milk samples were available. Because the infant was fed breast milk for ~1 month, infectious transmission via exposure to maternal milk cannot be ruled out. The patient also received multiple transfusions with blood products, but all were confirmed to be negative for CMV.

Regardless of mode and timing of acquisition, the concurrent diagnoses of CMV disease and NEC allow speculation about causality. Although CMV enteritis often occurs in immunocompromised adults, it is uncommon in the neonate3 and rarely presents with such extensive pneumatosis intestinalis as found in our patient.3,16 Most reported cases survived.

The concomitant finding of CMV inclusion bodies and genetic sequence evidence for Proteobacteria predominance raises interesting questions about viral-bacterial interactions. It is currently unknown what role CMV plays in the development of NEC. In the adult patient with inflammatory bowel disease, concomitant CMV infection is associated with exacerbated disease severity and treatment resistance.17,18 Animal models19 and clinical reports18 suggest that CMV may increase vulnerability to bacterial invasion and infection.
exaggerate intestinal immune response. It is particularly interesting that inflammation potentiation may occur without viral (re)activation. Proposed mechanisms include increased intestinal mucosal permeability, enhanced proinflammatory mediator production, and disruption of normal host-commensal interactions.

Premature infants have both diminished gut barrier function and an immature immune system. Immature secretion and absorption mechanisms reduce intestinal ability to remove pathogens and toxins. Decreased mucin production enhances bacterial adherence, while reduced defensin expression diminishes antimicrobial activity. Increased toll-like receptor 4 and decreased nuclear factor kappa-light-chain-enhancer of activated B cells inhibitor and acetylhydrolase expression in the premature gut promote inflammation. In this setting, any additional increase in gut permeability or stimulation of proinflammatory mediators may predispose to the development of NEC.

In our patient, we speculate that repeated antimicrobial courses may have selected for Gram-negative organisms. Proteobacteria predominance was roughly correlated with prolonged administration of broad-spectrum antibiotics (Table 1). CMV disease may have facilitated both bacterial invasion and an exaggerated proinflammatory response, resulting in fulminant NEC. It is unclear why no CMV DNA was found in the stool 2 days before the development of NEC. Relatively few CMV inclusion bodies were found in the intestine on postmortem histologic analysis. These findings and evidence of extensive bacterial invasion and pneumatosis intestinalis suggest that CMV may have played a facilitating rather than a causative role.

### Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>SWDI</th>
<th>Proteobacteria, %</th>
<th>Firmicutes, %</th>
<th>Other Phyla, %</th>
<th>Family</th>
<th>Genus</th>
<th>CMV-PCR</th>
<th>Feeding</th>
<th>Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antemortem fecal samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOL 10</td>
<td>0.345</td>
<td>0.7</td>
<td>76.6</td>
<td>2.9</td>
<td>94.5</td>
<td>NPO</td>
<td>+</td>
<td>On vanc/cftz (2 d after amp/gent)</td>
<td></td>
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<tr>
<td>DOL 12</td>
<td>1.800</td>
<td>76.6</td>
<td>14.7</td>
<td>8.7</td>
<td>74.4%</td>
<td>NPO</td>
<td>+</td>
<td>On vanc/cftz (4 d after amp/gent)</td>
<td></td>
</tr>
<tr>
<td>DOL 19</td>
<td>2.448</td>
<td>46.8</td>
<td>29.9</td>
<td>23.5</td>
<td>Enterobacteriaceae, 94.5%</td>
<td>Formula, PPO</td>
<td>Fluconazole prophylaxis, no antibiotics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOL 25</td>
<td>0.413</td>
<td>0.02</td>
<td>9.8</td>
<td>90.2</td>
<td>Unclassified, 72.1%</td>
<td>Bacteroidaceae</td>
<td>Fluconazole prophylaxis, no antibiotics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOL 39</td>
<td>0.579</td>
<td>97.5</td>
<td>0.9</td>
<td>2.4</td>
<td>Enterobacteriaceae, 97.5%</td>
<td>Formula, PPO</td>
<td>No antibiotics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOL 46</td>
<td>0.067</td>
<td>99.6</td>
<td>0.4</td>
<td>0</td>
<td>Enterobacteriaceae, 99.6%</td>
<td>Formula, PPO</td>
<td>No antibiotics</td>
<td></td>
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<tr>
<td>Postmortem samples</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileal fecal sample</td>
<td>0.057</td>
<td>99.9</td>
<td>0</td>
<td>0</td>
<td>Klebsiella, 99.9%</td>
<td>No</td>
<td>Formular, PPO</td>
<td>Breast milk, FPO</td>
<td>Fluconazole prophylaxis, no antibiotics</td>
</tr>
<tr>
<td>Ileal mucosal scraping</td>
<td>0.045</td>
<td>99.9</td>
<td>0</td>
<td>0</td>
<td>Klebsiella, 99.9%</td>
<td>No</td>
<td>Formular, PPO</td>
<td>Breast milk, FPO</td>
<td>Fluconazole prophylaxis, no antibiotics</td>
</tr>
</tbody>
</table>

Amp, ampicillin; cftz, ceftazidime; FPO, full enteral feeds; gent, gentamicin; ND, not detected; NPO, no enteral feeds; PPO, partial enteral feeds; SWDI, Shannon-Weaver Diversity Index; vanc, vancomycin.
CMV infection in our patient may have been a serendipitous finding, unrelated to the development of NEC; however, the speculation that CMV and perhaps other viruses may predispose to NEC is an interesting hypothesis that deserves further research. Practitioners should consider including CMV disease in their differential diagnosis of an infant with recurrent feeding intolerance and NEC. Antiviral therapy for neonatal CMV is controversial, but it may be considered in a severely symptomatic infant.

REFERENCES

10. Wang Y, Hoenig JD, Malin KJ, et al. 16S rRNA gene-based analysis of fecal microbiota and perhaps other viruses may predispose to NEC is an interesting hypothesis that deserves further research. Practitioners should consider including CMV disease in their differential diagnosis of an infant with recurrent feeding intolerance and NEC. Antiviral therapy for neonatal CMV is controversial, but it may be considered in a severely symptomatic infant.

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