Pathology and Pathogenesis of Fatal *Bordetella pertussis* Infection in Infants

Christopher D. Paddock,1 Gary N. Sanden,2 James D. Cherry,3 Anthony A. Gal,4 Claire Langston,6 Kathleen M. Tatti,2 Kai-Hui Wu,7 Cynthia S. Goldsmith,1 Patricia W. Greer,1 Jeltley L. Montague,1 Mark T. Eliason,1 Robert C. Holman,2 Jeannette Guarner,1 Wun-Ju Shieh,1 and Sherif R. Zaki1

1Infectious Disease Pathology Branch and 2Office of the Director, Division of Viral and Rickettsial Diseases, and 3Meningitis and Vaccine-Preventable Diseases Branch, Centers for Disease Control and Prevention, and 4Department of Pathology, Emory University School of Medicine, Atlanta, Georgia; 5Department of Pediatrics, David Geffen School of Medicine, University of California, Los Angeles; and 6Department of Pathology, Texas Children’s Hospital and Baylor College of Medicine, Houston

**Background.** Each year, *Bordetella pertussis* infection causes an estimated 294,000 deaths worldwide, primarily among young, nonvaccinated children. Approximately 90% of all deaths due to pertussis in the United States occur in young infants. These children often develop intractable pulmonary hypertension; however, the pathophysiologic mechanism responsible for this complication has not been well characterized, and there have been no detailed descriptions of the pathology of this disease since the 1940s.

**Methods.** Respiratory tissue samples obtained at autopsy from 15 infants aged ≤4 months who had polymerase chain reaction– or culture-confirmed *B. pertussis* pneumonia were evaluated by multiple histochemical stains, immunohistochemical evaluation, and electron microscopic examination.

**Results.** The pulmonary histopathologic examination of the samples revealed a descending infection dominated by necrotizing bronchiolitis, intra-alveolar hemorrhage, and fibrinous edema. All samples had marked leukocytosis, and most showed luminal aggregates of abundant leukocytes in small pulmonary arteries, veins, and lymphatics. A novel immunohistochemical stain for *B. pertussis* revealed abundant extracellular bordetellae in cilia of the trachea, bronchi, and bronchioles, as well as intracellular bacteria and antigens in alveolar macrophages and ciliated epithelium.

**Conclusions.** Pertussis should be suspected in any infant death associated with marked leukocytosis, bronchopneumonia, or refractory pulmonary hypertension, particularly in children aged ≤4 months. The pathologic findings identified in the respiratory tracts of these children, in addition to recognized physiologic responses of the infant lung to hypoxia, suggest that *B. pertussis* pneumonia triggers a cascade of events that includes acute pulmonary vasoconstriction and pertussis toxin–mediated increases in circulating leukocyte mass. These responses ultimately compromise pulmonary blood flow, exacerbate hypoxemia, and create a vicious cycle of refractory pulmonary hypertension.

Globally, pertussis is ranked among the 10 leading causes of childhood mortality and causes an estimated 294,000 pediatric deaths each year, predominantly among young nonvaccinated children [1]. Rates of reported infant pertussis and mortality associated with *Bordetella pertussis* infection have increased in the United States and in other industrialized nations during the past several decades [2–5], and 174 (86%) of the 203 deaths due to pertussis that were reported to the Centers for Disease Control and Prevention during the period 1990–2004 occurred in children aged <4 months [2, 6]. The disproportionate severity of *B. pertussis* infection in young children has been recognized for >100 years [7, 8]; however, the most catastrophic clinical complication of pertussis in infants, intractable pulmonary hypertension, was not reported until 1993 [9]. Refractory pulmonary hypertension, leading to cardiac...
failure and shock, is now recognized as a frequent problem in infants with fatal pertussis [2, 10–12].

For a life-threatening disease of this magnitude, there are surprisingly few contemporary reports of the histopathologic characteristics of fatal pertussis and none that effectively explain the pathophysiologic characteristics of B. pertussis–mediated pulmonary hypertension in young infants. There have been no extensive descriptions of the pathology of B. pertussis infection since the early 1940s [8, 13], and no detailed studies have documented the distribution of B. pertussis in respiratory tissues of humans with use of immunohistochemical (IHC) staining methods. Here, we report the pulmonary histopathologic characteristics of fatal infantile pertussis and use IHC techniques as a tool to provide insights into the complex pathogenesis of this infection.

METHODS

Histopathologic and IHC stains. Formalin-fixed, paraffin-embedded respiratory tissue specimens, obtained at autopsy from 15 infants for whom pertussis or another infectious cause of fatal pneumonia was suspected on the basis of compatible clinical features of the illness or histopathologic findings identified at autopsy, were submitted to the Centers for Disease Control and Prevention (Atlanta, GA) for diagnostic evaluation by medical examiners, community-based pathologists, infectious diseases clinicians, and public health epidemiologists. Some clinical and demographic information accompanied the specimens, although the completeness of these data varied considerably among the evaluated patients.

Sections (thickness, 3 μm) were stained with hematoxylin and eosin, Brown and Brenn, Brown-Hopps, Wolloch’s Giemsa, Verhoeff elastic, and Steiner silver stains and were tested specifically for B. pertussis with use of an immunoalkaline phosphatase technique. The primary monoclonal antibody (1H2; isotype IgG2a) used in the IHC assay reacts with the lipoooligosaccharide A of B. pertussis and was used at a dilution of 1:1000. The specificity of this antibody was evaluated with use of formalin-fixed, paraffin-embedded culture specimens of 4 geographically and temporally distinct strains of B. pertussis (E144, E161, E194, and D946), 4 strains of Bordetella bronchi-septica, and 5 strains each of Bordetella parapertussis and Bor-detella holmesii. Additional controls included formalin-fixed lung tissue specimens from patients with confirmed bacterial pneumonia caused by Streptococcus pneumoniae, Haemophilus influenzae, Legionella pneumophila, Neisseria meningitidis, Klebsiella pneumoniae, Streptococcus pyogenes, or Staphylococcus aureus. Representative sections of respiratory tissue from each B. pertussis–infected infant were also evaluated by IHC staining for S. pyogenes, S. aureus, N. meningitidis, H. influenzae, influenza A and B viruses, adenoviruses, respiratory syncytial virus, and cytomegalovirus [14]. The occurrence of each histopathologic feature was compared between the group of infants with B. pertussis as their only identified infecting pathogen and infants with infection due to B. pertussis and ≥1 other pathogen with use of the 2-sided Fisher’s exact test. A P value <.05 was considered to be statistically significant.

Molecular analyses. DNA was extracted from one 10-μm section of lung or upper airway tissue from each patient [15] and was eluted in a final volume of 100 μL. Ten microliters of extract was evaluated by a conventional PCR assay that amplifies a segment of the IS481 sequence of B. pertussis with use of primers IS481F and IS481R [15]. A real-time PCR assay was also used to amplify a 55–base pair segment of the B. pertussis toxin gene (ptxS1). The amplification mixture for the real-time assay contained 3 μL of extracted DNA in a 17-μL mixture containing 700 nmol each of primers 402U16 and 442L15, 300 nmol of probe 419U22P, and 0.8 μL of dimethylsulfoxide (K.-H.W., unpublished data). Tissue extracts were also evaluated for DNA of B. parapertussis with a real-time PCR assay designed to amplify a segment of the IS1001 sequence with use of primers Bpp-F1 and Bpp-R1 [16]. All amplicons of the appropriate sizes were sequenced. Sequence similarities were determined by the BLAST program.

Electron microscopy. Lung tissue samples were excised from paraffin blocks with use of a 2-mm punch, were deparaffinized in xylene at 60°C, were hydrated through a graded series of alcohols, and were fixed in buffered 2.5% glutaraldehyde and 1% osmium tetroxide. Fixed specimens were dehydrated through a graded series of alcohols and propylene oxide and were embedded in a mixture of Epon substitute and Araldite. Sections were stained with uranyl acetate and lead citrate.

RESULTS

Patient characteristics. The tissue samples that were evaluated were obtained from 7 girls and 8 boys from 8 US states, Costa Rica, and El Salvador who died of infection due to B. pertussis during the period 1984–2007 (table 1). All were infants aged ≤4 months. On the basis of the results of PCR assays performed at the Centers for Disease Control and Prevention (see the “Molecular analyses” section), all patients fulfilled the Council of State and Territorial Epidemiologists’ case definition for confirmed B. pertussis infection [6]. Five patients also had B. pertussis isolated by culture or PCR amplification of B. pertussis DNA that was performed at outside laboratories before or during the Centers for Disease Control and Prevention evaluation. An epidemiologic link to a family contact with confirmed or probable pertussis was described for 4 patients (table 1).

Eight (80%) of 10 patients for whom a temperature was reported had low-to-moderate fever (temperature range, 37.5°C–39.1°C). Eleven (85%) of 13 patients for whom WBC counts
Table 1. Demographic and clinical characteristics of infants with fatal *Bordetella pertussis* pneumonia, evaluated by histopathologic, immunohistochemical, and molecular techniques at the Centers for Disease Control and Prevention.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, weeks</th>
<th>Sex</th>
<th>State or country of residence</th>
<th>Year of death</th>
<th>Supportive laboratory or epidemiologic data for <em>B. pertussis</em> infectiona</th>
<th>Time from symptom onset to death, days</th>
<th>Peak leukocyte count, cells/L</th>
<th>Lymphocytes, %</th>
<th>Neutrophils, %</th>
<th>Monocytes, %</th>
<th>Radiographic evidence of pneumonia</th>
<th>Respiratory failureb</th>
<th>Shock</th>
<th>Pulmonary hypertensionc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>M</td>
<td>Texas</td>
<td>1984</td>
<td>DFA</td>
<td>12</td>
<td>75,000</td>
<td>45</td>
<td>38</td>
<td>12</td>
<td>Yes</td>
<td>Yes</td>
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<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>F</td>
<td>Texas</td>
<td>1997</td>
<td>None</td>
<td>7</td>
<td>90,000</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>F</td>
<td>Costa Rica</td>
<td>2001</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>F</td>
<td>Texas</td>
<td>2002</td>
<td>Culture</td>
<td>6</td>
<td>84,500</td>
<td>38</td>
<td>50</td>
<td>7</td>
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<td>Yes</td>
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<tr>
<td>5</td>
<td>4</td>
<td>F</td>
<td>Texas</td>
<td>2002</td>
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<td>5</td>
<td>39,000</td>
<td>53</td>
<td>40</td>
<td>3</td>
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</tr>
<tr>
<td>6</td>
<td>4</td>
<td>M</td>
<td>Michigan</td>
<td>2003</td>
<td>Epi link</td>
<td>16</td>
<td>75,200</td>
<td>49</td>
<td>26</td>
<td>6</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>F</td>
<td>Massachusetts</td>
<td>2003</td>
<td>Epi link</td>
<td>16</td>
<td>83,600</td>
<td>28</td>
<td>59</td>
<td>8</td>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>8</td>
<td>12</td>
<td>M</td>
<td>Oregon</td>
<td>2003</td>
<td>Epi link</td>
<td>7</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>M</td>
<td>Mississippi</td>
<td>2004</td>
<td>PCR</td>
<td>18</td>
<td>69,600</td>
<td>34</td>
<td>23</td>
<td>13</td>
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<td>Yes</td>
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<td>Yes</td>
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<tr>
<td>10</td>
<td>4</td>
<td>F</td>
<td>West Virginia</td>
<td>2004</td>
<td>Epi link, PCR</td>
<td>6</td>
<td>104,100</td>
<td>26</td>
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<td>NA</td>
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<td>Yes</td>
<td>Yes</td>
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<tr>
<td>11</td>
<td>7</td>
<td>M</td>
<td>El Salvador</td>
<td>2004</td>
<td>None</td>
<td>8</td>
<td>117,600</td>
<td>18</td>
<td>82</td>
<td>0</td>
<td>NA</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>12</td>
<td>4</td>
<td>M</td>
<td>El Salvador</td>
<td>2005</td>
<td>None</td>
<td>7</td>
<td>70,500</td>
<td>67</td>
<td>33</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>13</td>
<td>4</td>
<td>M</td>
<td>El Salvador</td>
<td>2005</td>
<td>None</td>
<td>14</td>
<td>34,600</td>
<td>73</td>
<td>25</td>
<td>NA</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>14</td>
<td>4</td>
<td>M</td>
<td>Oregon</td>
<td>2005</td>
<td>DFA, culture, PCR</td>
<td>9</td>
<td>81,100</td>
<td>54</td>
<td>23</td>
<td>15</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>M</td>
<td>New York</td>
<td>2007</td>
<td>PCR</td>
<td>3</td>
<td>85,600</td>
<td>54</td>
<td>NA</td>
<td>NA</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**NOTE.** DFA, direct fluorescent antibody; NA, data not available.

*a* Diagnostic techniques performed before specimen evaluation at the Centers for Disease Control and Prevention.

*b* Patients with documented receipt of mechanical ventilation.

*c* Pulmonary hypertension documented by echocardiography.
were available had peak WBC counts \( \geq 7 \times 10^9 \) cells/L. Radiographic evidence of pneumonia was reported for 8 patients. Ten patients were intubated and received mechanical ventilation for respiratory failure. Severe systemic hypotension was reported for 11 patients, and pulmonary hypertension was specifically documented for 5 patients. The median duration from symptom onset to death was 8 days.

**Histopathologic characteristics.** Sections of trachea from 9 infants were available, and most showed damaged mucosa with extensive loss of cilia, denuded and attenuated epithelium, and moderate-to-marked squamous metaplasia (table 2). Only 1 infant had prominent inflammatory infiltrates, composed of lymphocytes, macrophages, and plasma cells. Bronchial samples from 11 infants showed transmural and predominantly monocellular inflammatory infiltrates, extensively denuded epithelium, squamous metaplasia, and submucosal hemorrhages. Lung sections from 14 infants showed multifocal pulmonary hemorrhages and necrotizing bronchiolitis characterized by partial-to-complete luminal occlusion of bronchioles, terminal bronchioles, and respiratory bronchioles by varying amounts of necrotic debris, inflammatory cells, and denuded epithelium (figure 1A). In most samples, abundant coccobacillary bacteria, evident by hematoxylin and eosin stain, were situated in the cilia of respiratory epithelial cells lining the airways (figure 1B).

Approximately 75% of the samples showed focal or diffuse bronchopneumonia, and almost all had intra-alveolar collections of macrophages (figure 1C) and coarse, amphophilic, fibrinous edema (figure 1D). Nine (64%) of the 14 samples had hyaline membranes in alveoli indicative of diffuse alveolar damage. Partially occlusive fibrin thrombi, occurring generally with diffuse alveolar damage, were identified in a few small pulmonary arteries and arterioles of approximately one-half of the samples, but no conspicuous reduplication of the internal elastic lamina in these vessels was identified by elastic stain. Prominent edema and lymphangiectasia were commonly identified in the adventitia of pulmonary arteries and arterioles and in the interlobular septa and visceral pleura of most patients. Aggregates of granulocytes, lymphocytes, monocytes, and large

<table>
<thead>
<tr>
<th>Anatomic site, histopathologic feature</th>
<th>Infection due to <em>B. pertussis</em> only ((n = 9))</th>
<th>Coinfection with <em>B. pertussis</em> and (\geq 1) other pathogen ((n = 6))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Airways</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tracheitis</td>
<td>1/3</td>
<td>0/6</td>
</tr>
<tr>
<td>Damaged tracheal epithelium(^c)</td>
<td>2/3</td>
<td>5/6</td>
</tr>
<tr>
<td>Bronchitis</td>
<td>6/6</td>
<td>6/6</td>
</tr>
<tr>
<td>Necrotizing bronchiolitis</td>
<td>8/8</td>
<td>6/6</td>
</tr>
<tr>
<td>Alveoli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-alveolar hemorrhage</td>
<td>8/8</td>
<td>6/6</td>
</tr>
<tr>
<td>Fibrinous intra-alveolar edema</td>
<td>7/8</td>
<td>6/6</td>
</tr>
<tr>
<td>Macrophage-rich alveolar infiltrates</td>
<td>7/8</td>
<td>6/6</td>
</tr>
<tr>
<td>Neutrophilic bronchopneumonia</td>
<td>7/8</td>
<td>4/6</td>
</tr>
<tr>
<td>Diffuse alveolar damage</td>
<td>6/8</td>
<td>3/6</td>
</tr>
<tr>
<td>Pleura and septa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pleural edema or hemorrhage</td>
<td>6/8</td>
<td>4/6</td>
</tr>
<tr>
<td>Interlobular septal edema</td>
<td>6/8</td>
<td>5/6</td>
</tr>
<tr>
<td>Blood vessels and lymphatics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphangiectasia</td>
<td>7/8</td>
<td>6/6</td>
</tr>
<tr>
<td>Angiolympheatic leukocyte aggregates</td>
<td>7/8</td>
<td>5/6</td>
</tr>
<tr>
<td>Fibrin thrombi</td>
<td>5/8</td>
<td>3/6</td>
</tr>
</tbody>
</table>

\(^a\) The occurrence of each of the features did not differ statistically significantly between the 2 groups by the 2-sided Fisher’s exact test.

\(^b\) Coinfecting pathogens and viruses confirmed by culture, immunohistochemical stain, or rapid antigen detection included cytomegalovirus (1 patient), respiratory syncytial virus (2), influenza A virus (1), *Streptococcus pneumoniae* (2), *Streptococcus pyogenes* (2), *Moraxella (Branhamella) catarrhalis* (1), and viridans streptococci (1).

\(^c\) Characterized as loss or attenuation of cilia, denuded epithelium, or squamous metaplasia of respiratory epithelial cells.
immature leukocytes were identified in pulmonary arterioles, small arteries, and venules (figure 1E and 1F) and in dilated lymphatics in the interlobular septa and visceral pleura of most patients. Clusters of innumerable intra- and extracellular cocobacilli were identified in the bronchiolar and alveolar infiltrates by >1 histochemical stains for bacteria. These minute bacteria were more easily detected by Steiner and Giemsa stains than by Brown and Brenn or Brown-Hopps stains (figure 2).
**IHC assay.** The IHC assay reacted with all evaluated strains of *B. pertussis* and *B. bronchiseptica* but with none of the *B. parapertussis* or *B. holmesii* strains or the other bacterial organisms that caused the studied cases of pneumonia. Bordetellae were easily identified by IHC staining in the cilia of columnar epithelial cells in the trachea, bronchi, or bronchioles of all patients in this series (figure 3A–3C). These bacteria were occasionally found on ciliated cells lining the secretory ducts of the larynx and trachea and were found only once in mucous gland epithelium. Bordetellae were not attached to metaplastic or squamous epithelium in any of the involved airways. Most patients had abundant extracellular bordetellae and bacterial antigens in the alveoli (figure 3D). Bacteria were also identified in the cytoplasm of alveolar macrophages (figure 3E) and in columnar epithelial cells lining the airways (figure 3F).

IHC stains for other respiratory pathogens identified infections due to cytomegalovirus in 1 patient and *S. pyogenes* in 2 patients. During cultures of lung tissue samples obtained at autopsy, *S. pneumoniae* (2 patients), *Moraxella* (Branhamella) *catarrhalis* (1 patient), and viridans streptococci (1 patient) were isolated. Rapid antigen tests performed before death identified influenza A virus in 1 patient and respiratory syncytial virus in 2 patients. Except for numerous cytomegallic inclusions in the pneumocytes of 1 patient coinfected with cytomegalovirus, there were no significant differences in the dominant pulmonary histopathologic findings observed between patients infected with *B. pertussis* only and those coinfected with *B. pertussis* and another pathogen (table 2).

**Molecular analyses.** PCR assays amplified 181–base pair and 55–base pair segments of the *IS481* and *ptxS1* genes of *B. pertussis*, respectively, from formalin-fixed lung or trachea sections from all patients. There was no molecular evidence of infection due to *B. parapertussis* in respiratory tissue samples from any of the 15 patients.

**Electron microscopy.** Intact bacterial cells with characteristic gram-negative morphology, compatible with *Bordetella* species, were identified by electron microscopy in cilia of columnar epithelial cells lining the airways (figure 4A and 4B) and occasionally in the cytoplasm of these cells (figure 4C and 4D). Abundant intact bordetellae were also seen in the cytoplasm of alveolar macrophages (figure 4E and 4F).

**DISCUSSION**

The lungs of each child in this series exhibited consistent histopathologic features of a descending respiratory infection characterized by necrotizing bronchiolitis, bronchopneumonia, pulmonary hemorrhage and edema, and angiolymphatic leukocytosis. A novel IHC stain for *B. pertussis* revealed abundant intra- and extracellular bordetellae in airways and airspaces. To our knowledge, IHC staining of *B. pertussis* in human tissue samples has been reported only once, in a brief case report that presented results from use of an anti-pertactin monoclonal antibody to *B. pertussis* [17]. Coinfections with other viral or bacterial pathogens were reported or identified in several infants from our series, but the dominant histopathologic findings in the respiratory tissue samples from these patients were indistinguishable from those observed in the infants infected with *B. pertussis* only. Coinfections with *B. pertussis* and *B. parapertussis* have been reported [18, 19]; however, we did not identify this coinfection in any of the patients in this series.

Definite or presumptive clinical evidence of pulmonary hypertension was described for ~80% of the children whose tissue...
Figure 3. Immunohistochemical staining of *Bordetella pertussis* bacteria in the airways and airspaces of infants with pertussis with use of immunoalkaline phosphatase with naphthol-fast red and hematoxylin counterstain. Tangles of *B. pertussis* (red) in the cilia of columnar epithelial cells lining the trachea (A; original magnification, ×50) and bronchioles (B; original magnification, ×158) can be seen. C. Bacterial cells mixed with necrotic debris, mucus, and inflammatory cells, partially occluding the lumen of a small bronchiole (original magnification, ×50). D and E, Abundant intra- and extracellular bacteria in alveolar infiltrates (original magnification for D and E, ×12.5 and ×158, respectively). F, Intracellular bordetellae and bacterial antigens in the columnar epithelium of a bronchiole (original magnification, ×158). G, *Bordetella* antigens in the cytoplasm of a tracheal epithelial cell in an infant given treatment for pertussis (68 days after onset of symptoms and 57 days after isolation of *B. pertussis* by culture; original magnification, ×158).
samples were evaluated and may have been present in some or all of the remaining 20% for whom specific clinical data were not provided or the condition was undiagnosed. For >70 years, physicians have recognized an association between the magnitude of the leukocyte count and the severity of pertussis, noting that the highest leukocyte counts occur in the patients with pertussis pneumonia [20–22]. More recently, clinicians have recognized a strong association between extreme leukocytosis and pulmonary hypertension in infants with pertussis [10, 12, 23]. Median or mean leukocyte counts $>60 \times 10^9$ cells/L have been reported in several contemporary series involving children with fatal pertussis, and pneumonia was documented in 90%–100% of the deaths in these recent series [2, 4, 24, 25]. An adenosine diphosphate–ribosylating toxin (pertussis toxin) elaborated by B. pertussis elicits a dose-dependent leukocytosis in infected hosts [26], and our observations suggest that the extreme leukocytosis that accompanies B. pertussis pneumonia in infants results from enormous numbers of bordetellae that proliferate in the airspaces of these very young patients.

Increases in leukocyte mass can diminish blood flow by increasing vascular resistance; nonetheless, pulmonary hypertension is generally initiated and maintained by many factors, and it is likely that other physiologic circumstances acting in concert with profound leukocytosis are involved in the evolution of this process in fatal infant pertussis. The pulmonary vasculature of infants is highly reactive during the first few months after birth, when the muscular component of small pulmonary arteries is relatively prominent. Hypoxemia, particularly when coupled with acidosis, triggers marked pulmonary vasoconstriction [27], and several types of acute infectious pneumonia...
can initiate varying degrees of hypoxia-induced pulmonary hypertension in the infant lung [28]. Nonetheless, pertussis pneumonia appears to be distinct in its ability to cause severely elevated arterial pressures in the infant lung that are intractable to conventional therapies.

Young infants infected with *B. pertussis* exhibit abnormally prolonged apneic pauses that result in frequent decreases in arterial oxygen saturation and severe hypoxia during continued breathing movements [29]. Apnea or cyanosis is documented for ~70% of infants who die of pertussis [2], and in several studies involving children with severe pulmonary hypertension associated with pertussis, nonsurvivors had significantly lower arterial blood pH values than did survivors [11, 12]. Collectively, these data suggest that *B. pertussis* pneumonia may trigger a cascade of events that includes hypoxia, acidosis, acute pulmonary vasoconstriction, and pertussis toxin–mediated increases in circulating leukocyte mass that combine to restrict pulmonary blood flow (figure 5). These compounded effects produce markedly elevated pressures in the vasculature of the infant lung that exacerbate hypoxemia and ultimately cause cardiac failure, shock, and acute respiratory distress. These conditions, in turn, aggravate the preexistent hypoxic vasoconstriction and create a vicious cycle of refractory pulmonary hypertension. The pathophysiologic mechanism of pulmonary hypertension in infants with severe *B. pertussis* infection remains hypothetical; however, several recent reports that describe improvement of hypoxemia after exchange transfusion or leukopheresis to reduce leukocyte mass in infants with pertussis pneumonia [30–32] support this proposed mechanism.

Early 20th century descriptions of the histopathologic characteristics of pertussis emphasized large airway disease as the principal component of *B. pertussis* infection and documented masses of bordetellae in the cilia of the tracheal and bronchial mucosa, with damage to this epithelium [33]. Pathologists later reported a sequence of events for fatal pertussis in which endobronchitis and endobronchiolitis evolved to peribronchitis and peribronchiolitis and, eventually, to bronchopneumonia [13, 34]. Diffuse bronchopneumonia has been documented in most of the limited contemporary histopathologic descriptions of fatal pertussis [10, 17, 35, 36], and in our study and others, we identified *B. pertussis* distributed abundantly in alveolar macrophages that comprised the predominant inflammatory cell in the pulmonary infiltrates. Animal models of pertussis and in vitro studies using human monocytes have revealed that *B. pertussis* can enter, survive, and persist in macrophages for as long as 40 days and that this process may contribute significantly to the total bacterial load in the lungs [37–40]. During 1 microbiologic study of pertussis-related deaths in the early 1930s, *B. pertussis* was routinely found by culture of lung specimens from patients who died within 30 days after illness onset (G. M. Lawson, personal communication). The clinical and pathogenic consequences of intracellular bordetellae in patients with pertussis pneumonia are unknown; however, bacterial residence within macrophages may hinder the ability of some antibiotics to effectively accumulate to bactericidal levels in this intracellular compartment.

The persistence of bacterial antigens in airway epithelium may also contribute to the chronicity that characterizes the clinical syndrome of pertussis. We identified intact bordetellae in the cytoplasm of ciliated columnar epithelial cells, consistent with the findings from studies that have reported invasion of mouse ependymal cells and cultured human tracheal epithelial cells with *B. pertussis* [41–43]. Many of these bacteria are destroyed within several days; however, the fate of residual intracellular antigens has not been well characterized. Recently, we evaluated the tissue samples from a 4-month-old infant who died of causes other than pertussis but from whom *B. pertussis* had been yielded by a culture performed 8 weeks before death. In this patient, rare *Bordetella* antigens were stained in the cytoplasm of tracheal epithelial cells with use of the IHC assay (figure 3G), suggesting that bacterial antigens may persist in the upper respiratory tract for at least several weeks after antibiotic therapy.

Although current numbers of pertussis cases and deaths due to pertussis are a small fraction of the numbers observed during the prevaccine era [7, 44], morbidity and mortality attributable to *B. pertussis* remain underrecognized and underreported [45, 46]. The IHC and molecular assays that we describe provide methods to diagnose otherwise unexplained infant deaths or...
to confirm presumptive cases of fatal pertussis, particularly when samples for culture or PCR are not available. In this context, confirmation of fatal *B. pertussis* infection by IHC assay provides an opportunity to assess more accurately the magnitude and frequency of fatal pertussis in the infant cohort.

The paucity of contemporary pathologic descriptions of fatal pertussis reflects the triumph of effective vaccine strategies against this devastating childhood illness. Nonetheless, the recognized incidence of *B. pertussis* infection among US infants increased by 49% during the 1990s, compared with incidence during the 1980s [47]. It is also likely that many infant deaths attributable to pertussis, even among those children for whom autopsy data may be available, are missed, because many pathologists and medical examiners are unfamiliar with the clinico-pathologic features of fatal pertussis pneumonia.

Pertussis should be suspected in any infant death associated with marked leukocytosis, bronchopneumonia, or refractory pulmonary hypertension, particularly in children aged ≤4 months. The insights provided by pathologic diagnosis and evaluation of patients with fatal *B. pertussis* infection can help to sharpen the epidemiologic focus of infant pertussis, to unmask its elaborate pathogenesis, and to guide interventional approaches to combat this recalcitrant and life-threatening illness.

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