Polymorphism of Tumor Necrosis Factor-α and Risk and Severity of Bronchopulmonary Dysplasia Among Very Low Birth Weight Infants

S. Nadya J. Kazzi, MD, MPH*; U. Olivia Kim, MD*; Michael W. Quasney, MD, PhD‡; and Irina Buhimschi, MD§

ABSTRACT. Background. Preterm infants with bronchopulmonary dysplasia (BPD) exhibit prolonged elevation of inflammatory indices in their tracheal aspirates. Tumor necrosis factor-α (TNF-α) is a central mediator of the inflammatory response. The adenine-containing alleles of TNF-α−308 and lymphotoxin-α+250 have been associated with increased levels of TNF-α, whereas the adenine allele of TNF-α−238 produces lower levels of TNF-α after stimulation. High levels of TNF-α may promote chronic inflammation by overwhelming counter-regulatory mechanisms and may lead to the development of BPD. Low levels of TNF-α may decrease the risk and severity of BPD.

Objective. To determine whether alleles of TNF-α play a role in the susceptibility and/or severity of BPD among very low birth weight infants.

Methods. Infants with birth weights of ≤1250 g were included. Genotypic analyses (polymerase chain reaction-restriction fragment length polymorphism assays) were performed with DNA extracted from whole-blood samples.

Results. Infants who developed BPD (fraction of inspired oxygen at postconceptional age of 36 weeks of >0.21, n = 51) had a younger gestational age (mean ± SD: 27 ± 4 vs 29 ± 2 weeks) and lower birth weight (853 ± 184 vs 997 ± 193 g) than did infants without BPD (n = 69). The genotypic distributions of lymphotoxin-α+250 and TNF-α−308 were comparable among the groups of infants. However, the AA and GA TNF-α−238 genotypes were much less likely to occur among infants with BPD than among infants without BPD. The adenine allele of TNF-α−238 was absent among infants with severe BPD and occurred significantly less often among infants with moderate or severe BPD, compared with infants with mild BPD. The number of adenine alleles of TNF-α−238 was correlated inversely with the severity of BPD (r = −0.341).

Conclusion. The adenine allele of TNF-α−238 may reduce the risk and severity of BPD. Pediatrics 2004; 114:e243–e248. URL: http://www.pediatrics.org/cgi/content/full/114/2/e243; tumor necrosis factor-α, cytokines, bronchopulmonary dysplasia, polymorphism, low birth weight infants.

ABBREVIATIONS. TNF-α, tumor necrosis factor-α; VLBWI, very low birth weight infant; BPD, bronchopulmonary dysplasia; LT-α, lymphotoxin-α; PCA, postconceptional age.

Despite considerable obstetric and neonatal advances in the care of very low birth weight infants (VLBWIs), bronchopulmonary dysplasia (BPD) continues to occur among 25% to 30% of surviving infants. The disease has been recognized as a significant risk factor for neurodevelopmental morbidity. Several factors are thought to contribute to the pathogenesis of the disease, including lung immaturity, pulmonary barotrauma, oxygen toxicity, infection, and pulmonary inflammation. Prenatal exposure to stimuli of inflammation, including postnatal lung infections, coupled with repetitive positive pressure inflation and oxygen toxicity induces a complex inflammatory response in the airways and interstitium of immature lungs, leading to the structural changes observed among infants with BPD. Investigators have documented the presence of several markers of inflammation in the airway lavage fluid of infants who develop BPD. Feedback mechanisms that regulate the secretion and effects of proinflammatory cytokines are vital in containing the initial inflammatory response and initiating repair processes. An exaggerated proinflammatory response may overwhelm counter-regulatory mechanisms, amplify the inflammatory process, and result in tissue autoinjury and structural changes. One of the earliest proinflammatory cytokines produced during an inflammatory response is tumor necrosis factor-α (TNF-α). After its secretion by activated monocytes and alveolar macrophages, TNF-α triggers the release of secondary mediators of inflammation, such as interleukin-1, interleukin-6, and several others, as well as of itself. Individual differences in the production of TNF-α were reported previously. TNF-α expression is thought to be regulated at the transcriptional level. A number of single-nucleotide polymorphisms have been identified within the TNF gene promoter locus on chromosome 6. More specifically, substitution of the nucleotide adenine for guanine at positions TNF-β+250 (also referred to as lymphotoxin-α [LT-α]+250) and TNF-α−308 have been associated with increased levels of production of TNF-α. Such elevated levels, even if low, may be magnified several-fold in vivo, because TNF-α exerts positive feedback on its own secretion. The process may result in an uncon-
trolled inflammatory response, triggering progressive tissue injury. Another polymorphism resulting from the substitution of an adenine for a guanine moiety occurs at position −238, within the TNF-α promoter region. The adenine-containing allele of TNF-α−238 has been associated with decreased production of TNF-α, reduced severity of nonpulmonary diseases, and improved outcomes among adults. The objectives of our study were to examine the effects of LT-α+250, TNF-α−308, and TNF-α−238 on the susceptibility to and/or severity of BPD among VLBWIs.

METHODS

Patients

Preterm infants with a body weight of ≤1250 g were recruited from the neonatal intensive care nursery at Hutzel Women’s Hospital (Detroit, MI) between July 1, 2001, and July 31, 2003. After informed consent was obtained, 0.5 mL of whole blood either was drawn from an indwelling arterial or venous umbilical catheter or was obtained by venipuncture. Genotypic analysis of TNF-α polymorphic sites was performed by using a polymerase chain reaction, followed by restriction enzyme digestion of DNA extracted by using a genomic DNA purification kit (Promega, Madison, WI).

Gestational age was determined from the maternal last menstrual period and/or early prenatal ultrasonograms, when available. For pregnancies with limited or no prenatal care, gestational age was determined by averaging 2 estimates obtained from late prenatal ultrasonograms and postnatal assessments with the examination described by Ballard et al.

The severity of respiratory illness at the time of entry into the study was determined by the ventilatory index, defined as mean arterial pressure × fraction of inspired oxygen. The diagnosis of a patent ductus arteriosus was confirmed with a 2-dimensional Doppler flow echocardiographic study. Pneumonia (bacterial, vi- ruminal or ureaplasmal/mycoplasmal) was diagnosed on the basis of positive tracheal aspirate culture, enzyme-linked immunosorbent assay, or other diagnostic test results obtained because of deterioration of the infant’s respiratory status, including initiation of treatment with antimicrobial agents and/or increasing respiratory support with positive findings on chest radiographs. Late-onset sepsis was diagnosed on the basis of positive blood culture results obtained after 5 days of age. Diagnosis and staging of necrotizing enterocolitis were based on the criteria described by Walsh and Kliegman.

BPD was defined as dependency on mechanical ventilation and/or supplemental oxygen at a postconceptional age (PCA) of 36 weeks, with radiographic evidence of parenchymal lung disease. The severity of BPD among surviving infants was determined as described by Jobe and Bancalari. Briefly, for infants with gestational ages of <32 weeks, the diagnosis of BPD was made if oxygen therapy was used for at least 28 days, with classification as follows: 1) mild BPD: infant was breathing room air at 36 weeks PCA or discharge, whichever came first; 2) moderate BPD: infant required <30% oxygen at 36 weeks PCA or discharge, whichever came first; 3) severe BPD: infant required ≥30% supplemental oxygen and/or positive pressure therapy (positive pressure ventilation or nasal continuous positive airway pressure therapy) at 36 weeks PCA or discharge, whichever came first.

Statistical Analyses

Statistical analyses were performed with SPSS 10.0 software (SPSS Inc, Chicago, IL). Student’s t tests, χ2 tests, or Fisher’s exact tests were used to compare continuous and categorical variables, as indicated. Kendall’s τ-b correlation was used to examine the relationship between the severity of BPD and grades of adenine alleles of TNF-α genotypes (AA = 2, GA = 1, GG = 0). Multivariate logistic regression analysis was performed to assess the contributions of TNF-α alleles and other independent risk factors to the outcomes. P ≤ .05 was chosen as the level of significance.

RESULTS

A total of 154 of 225 eligible infants were enrolled in the study. One hundred twenty infants survived to 36 weeks PCA. Infants who developed BPD had a lower birth weight and younger gestational age, were more likely to have been exposed to prenatal corticosteroids and prolonged rupture of the fetal membranes (≥18 hours), and were more likely to have Apgar scores at 5 minutes of ≤5 (P < .05) (Table 1). The incidence of respiratory distress syndrome, the number of doses of surfactant, the severity of respiratory illness (as indicated by the ventilatory index), and the incidences of pulmonary hemorrhage, patent ductus arteriosus, pneumonia, late-onset sepsis, and necrotizing enterocolitis (stage II or greater) were significantly greater among infants with BPD, compared with infants without BPD (P < .05) (Table 2). Similarly, infants with BPD were more likely to develop grade III/IV intraventricular hemorrhage and advanced stages (stage ≥3) of retinopathy of prematurity (P < .05) (Table 3). The frequencies of the variant adenine alleles of LT-α+250, TNF-α−238, and TNF-α−308 in our patient population were 0.57, 0.06, and 0.12, respectively. Similar frequencies of these adenine alleles of TNF-α among adults have been reported.

There was significant linkage disequilibrium between LT-α+250 and TNF-α−238 (P = .05) and between LT-α+250 and TNF-α−308 (P = .00) but not between TNF-α−308 and TNF-α−238 (P = .70) (Fig 1). There was no difference in the genotypic distributions of alleles of LT-α+250 and TNF-α−308 among groups of infants (Fig 2). However, infants with BPD were significantly less likely to carry the AA or GA TNF-α−238 genotype, compared with infants without BPD (χ2 test, P = .026) (Fig 2). None of the infants with BPD carried the AA TNF-α−238 genotype, compared with 3% of infants without BPD, and only 2% of infants with BPD carried the GA TNF-α−238 genotype, compared with 14% of infants without BPD (χ2 test, P < .05) (Fig 2). When we classified our infants according to the severity of BPD (mild: n = 24; moderate: n = 20; severe: n = 31), on the basis of the description by Jobe and Bancalari, our sample size increased (n = 75) because of inclusion of infants with mild BPD (who had required oxygen for ≥28 days but were breathing room air by 36 weeks). We found no dif-

<table>
<thead>
<tr>
<th>TABLE 1. Infant Characteristics</th>
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<tr>
<td><strong>BPD</strong></td>
</tr>
<tr>
<td><strong>n = 51</strong></td>
</tr>
<tr>
<td>Birth weight, g*</td>
</tr>
<tr>
<td>Gestational age, wk*</td>
</tr>
<tr>
<td>Gender, male/female‡</td>
</tr>
<tr>
<td>Race, white/African Americans/Hispanic‡</td>
</tr>
<tr>
<td>Maternal chorioamnionitis‡</td>
</tr>
<tr>
<td>Prolonged rupture of fetal membranes (≥18 h)‡</td>
</tr>
<tr>
<td>Prenatal corticosteroids‡</td>
</tr>
<tr>
<td>Cesarean birth‡</td>
</tr>
<tr>
<td>Apgar score at 5 min of ≤5‡</td>
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</tbody>
</table>

* Student’s t test. Values are mean ± SD.
† P < .05
‡ χ2 test. Values are number (percentage) of infants.
ventilation index

Infant Outcomes

TABLE 3.

<table>
<thead>
<tr>
<th></th>
<th>BPD (n = 51)</th>
<th>No BPD (n = 69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory distress syndrome*</td>
<td>46 (90%)</td>
<td>45 (65%)†</td>
</tr>
<tr>
<td>Doses of surfactant‡</td>
<td>3 ± 1</td>
<td>1 ± 1†</td>
</tr>
<tr>
<td>Ventilatory index§</td>
<td>1.5 (0–3.3)</td>
<td>1 (1–2.0)†</td>
</tr>
<tr>
<td>Pulmonary hemorrhage*</td>
<td>8 (16%)</td>
<td>3 (4%)†</td>
</tr>
<tr>
<td>Patent ductus arteriosus, indomethacin/ligation*</td>
<td>20 (39%) / 7 (14%)</td>
<td>17 (25%) / 3 (4%)†</td>
</tr>
<tr>
<td>Pneumonia*</td>
<td>23 (45%)</td>
<td>7 (10%)†</td>
</tr>
<tr>
<td>Late-onset sepsis*</td>
<td>24 (47%)</td>
<td>16 (23%)†</td>
</tr>
<tr>
<td>Necrotizing enterocolitis (stage II or greater)*</td>
<td>11 (22%)</td>
<td>4 (6%)†</td>
</tr>
</tbody>
</table>

* χ² test. Values are number (percentage) of infants.
† P < .05.
‡ Student’s t test. Values are mean ± SD.
§ Ventilatory index = mean airway pressure × fraction of inspired oxygen at entry into the study. Mann-Whitney test. Values are median (25–75% interquartile range).

BPD: bronchopulmonary dysplasia

TABLE 2. Hospital Course

<table>
<thead>
<tr>
<th></th>
<th>BPD (n = 51)</th>
<th>No BPD (n = 69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraventricular hemorrhage (grade III or IV)*</td>
<td>9 (18%)</td>
<td>4 (6%)†</td>
</tr>
<tr>
<td>Posthemorrhagic hydrocephalus*</td>
<td>7 (14%)</td>
<td>3 (4%)</td>
</tr>
<tr>
<td>Retinopathy of prematurity (stage ≥3)*</td>
<td>7/49 (14%)</td>
<td>3/55 (5%)†</td>
</tr>
<tr>
<td>Death at &gt;36 wk*</td>
<td>5 (10%)</td>
<td>1 (1%)</td>
</tr>
</tbody>
</table>

* χ² test. Values are number (percentage) of infants.
† P < .05.

It is well accepted that BPD among preterm infants results from multiple risk factors acting in concert to induce lung injury, by initiating what seems to be a protracted inflammatory response. Such a prolonged inflammatory process is thought to be attributable to an imbalance between proinflammatory and antiinflammatory mediators, which have been identified in the tracheal aspirates of infants who develop BPD. One mediator that plays a central role in the regulation of inflammation is TNF-α.* Excessive production of TNF-α has been implicated in several diseases characterized by chronic inflammation, including rheumatoid arthritis, Crohn’s disease, psoriasis, systemic lupus erythematosus, multiple sclerosis, and Alzheimer’s disease. Because chronic inflammation is a characteristic feature of the lungs of infants who develop BPD, we explored the effects of TNF-α-308 and LT-α+250 on the susceptibility to and severity of BPD. In this study, we found no difference in the genotypic distributions of alleles of LT-α+250 and TNF-α-308 among infants with or without BPD or among infants with BPD of varying severity.

Another allele of the TNF-α gene, ie, TNF-α-238, has been associated with lower levels of production of TNF-α after stimulation. We speculated that lower levels of TNF-α might be associated with less severe and/or briefer inflammation, possibly reducing the risk and/or severity of BPD. Our study showed that the frequency of the AA/GA TNF-α-238 genotype was significantly lower among infants who developed BPD, compared with infants without BPD; furthermore, these genotypes were absent among infants with severe BPD and occurred significantly less often among infants with moderate BPD, compared with infants with mild BPD. The severity of BPD was correlated significantly and inversely with the number of adenine alleles of TNF-α-238. Multiple logistic regression analysis revealed that pneumonia and the absence of an adenine allele of TNF-α-238 were highly predictive of the development of BPD.

Similar effects of the adenine allele of TNF-α-238 have been observed among adults. Lower frequencies of the adenine allele of TNF-α-238 were noted among patients with cancer (gastric carcinoma, uterine cervical carcinoma, colorectal carcinoma, and renal cell carcinoma), compared with healthy control subjects. Similarly, the frequencies of the adenine allele and the GA genotype of TNF-α-238 were significantly lower among patients with rheumatic heart disease, compared with healthy control subjects. Patients with rheumatoid arthritis and the GA TNF-α-238 genotype were less likely to exhibit radiographic evidence of joint erosions and demonstrated fewer erosions in their hand joints, compared with healthy control subjects. Similarly, the frequencies of the adenine allele and the GA genotype of TNF-α-238 were significantly lower among patients with rheumatic heart disease, compared with healthy control subjects. Patients with rheumatoid arthritis and the GA TNF-α-238 genotype were less likely to exhibit radiographic evidence of joint erosions and demonstrated fewer erosions in their hand joints, compared with healthy control subjects.
with patients with the GG TNF-α−238 genotype.21 Patients with multiple myeloma who were carriers of the TNF-α−238 adenine allele demonstrated a better rate of response to treatment with thalidomide and higher 12-month progression-free and overall survival rates, compared with patients with the TNF-α−238 guanine allele.22

The mechanism by which the adenine allele of TNF-α−238 decreases production of TNF-α remains unclear. However, it has been suggested that the presence of a repressor site identified in a 25-base pair stretch that includes the 238 position in the TNF-α gene promoter locus may suppress the transcription of TNF-α.35,36

Another possible explanation for our findings is that TNF-α−238 is in linkage disequilibrium with other genes that may be involved in the regulation of inflammation. The TNF locus is located within the major histocompatibility complex on chromosome 6. The region has a cluster of highly polymorphic genes and encodes several proteins that are involved in inflammation.37,38 It is possible that TNF-α−238 represents a marker for a cluster of neighboring genes that determine infants’ inflammatory responses and the risk and/or severity of diseases characterized by such responses. Although our patient population consisted of only VLBWIs, the finding of similar frequencies of TNF-α alleles among our infants and adults supports the hypothesis that our findings are related to the adenine allele of TNF-α−238 and/or its haplotypes.

Differences in the production of TNF-α among individuals are thought to be genetically determined.11,12 Production of TNF-α protein is regulated at the transcriptional and posttranscriptional levels.39 Transcriptional regulation of the TNF gene involves a highly complex, tightly controlled system that is stimulus and cell type specific.40 Therefore, elucidating the potential effects that TNF single-nucleotide polymorphisms may have on the transcriptional regulation of TNF is difficult, in addition to any role that single-nucleotide polymorphisms in
genes of cytokines involved in TNF-α production may have in determining the levels of TNF-α.

In a preliminary study in which we measured serial levels of TNF-α in bronchoalveolar lavage fluid of intubated preterm infants at risk of BPD, we found that TNF-α levels were elevated on day 7 among infants who developed BPD, compared with infants without BPD. Infants with AA or GA LT-α+250 genotypes demonstrated higher levels of TNF-α in their bronchoalveolar lavage fluid on day 7, compared with infants with the GG genotype. No significant differences in TNF-α levels were associated with the presence of adenine alleles of TNF-α−238 or TNF-α−308.

Most diseases are the result of an interplay between genetic and environmental factors. It is well accepted that the presence of certain genes may determine an infant’s susceptibility to and/or severity of certain illnesses. However, phenotypic expression of some genes may not occur in the absence of precipitating environmental factors.

CONCLUSIONS
Our findings suggest that the presence of the adenine allele of TNF-α−238 among VLBWIs may confer a protective effect against the development of BPD. Among infants who develop BPD, the presence of the adenine allele of TNF-α−238 seems to decrease the severity of the disease. Genotypic analysis of VLBWIs at risk of developing BPD may provide clinicians with a useful tool for targeting preventative therapies in the future.

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