MAJOR ARTICLE

Association between Common Toll-Like Receptor 4 Mutations and Severe Respiratory Syncytial Virus Disease

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Background. The clinical spectrum of respiratory syncytial virus (RSV) bronchiolitis in previously healthy infants is extremely variable. Thus, it is likely that factors such as genetic heterogeneity contribute to disease severity. Toll-like receptor 4 (TLR4) and CD14 are part of a receptor complex involved in the innate immune response to RSV.

Methods. The association of the TLR4 mutations (Asp299Gly and Thr399Ile) and the CD14/−159 polymorphism were analyzed in 99 infants hospitalized with severe RSV bronchiolitis (group I). Eighty-two ambulatory infants with mild RSV bronchiolitis (group II) and 90 healthy adults (group III) composed the 2 control groups. The TLR4 mutations and the CD14/−159 polymorphism were genotyped by use of reverse-transcriptase polymerase chain reaction and restriction fragment–length polymorphism analysis, respectively.

Results. Each of the TLR4 mutations, either alone or in cosegregation, were associated with severe RSV bronchiolitis: the Asp299Gly and Thr399Ile mutations were significantly overrepresented in group I, compared with groups II and III. No association between the CD14/−159 polymorphism and RSV bronchiolitis was found.

Conclusions. These findings suggest that TLR4 mutations, but not the CD14/−159 polymorphism, are associated with an increased risk of severe RSV bronchiolitis in previously healthy infants.

Bronchiolitis is an acute infection of the respiratory tract and is mainly associated with respiratory syncytial virus (RSV). The clinical course of RSV bronchiolitis is extremely variable, ranging from mild upper respiratory symptoms to severe respiratory distress and, occasionally, death [1]. Despite the fact that virtually all infants are infected by RSV by the age of 2 years, only 40%–50% develop involvement of the lower respiratory tract, and 1%–2% develop severe disease leading to hospitalization. Interestingly, 40%–60% of infants hospitalized with RSV bronchiolitis develop recurrent wheezing episodes through childhood. Although there are known risk factors associated with severe disease—such as prematurity, congenital heart disease, and chronic lung disease—the majority of the infected infants have no obvious risk factors [2]. Thus, it is likely that other factors, such as genetic heterogeneity of the host rather than the virus alone, contribute to disease severity.

It is currently well accepted that, in most infectious diseases caused by intracellular pathogens, a dominant Th2 cytokine response is associated with disease progression and a dominant Th1 cytokine production is protective. Therefore, it is assumed that the interplay between the 2 different responses is crucial for recovery from RSV bronchiolitis [1]. Indeed, several studies have shown a correlation between a predominant Th2 response in infants with RSV bronchiolitis and disease severity [3, 4]. Several recent studies have shown the association of interleukin (IL)–4 and IL-4 receptor α polymorphisms with severe RSV bronchiolitis [5, 6]. Other studies have shown that the presence of poly-

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morphisms in genes that are part of the innate immunity, such as surfactant protein (Sp) A and SpD, correlates with severe RSV bronchiolitis [7, 8].

In recent years, the role of innate immunity and its interaction with adaptive immunity has, largely, been explored. A major role of innate immunity is to sense pathogens and deflect the adaptive immunity into a Th1/Th2 response, through secretion of cytokines and interaction of ligands. The human Toll-like family of proteins, which consists of at least 10 members, has recently been identified. These proteins were found to be a critical link between immune stimulants produced by microorganisms and the initiation of host defense [9, 10]. TLR4 and CD14 have recently been shown to be major lipopolysaccharide (LPS) receptors. Mutations in mouse and human TLR4 were found to be associated with hyporesponsiveness to LPS and to confer an increased risk of gram-negative infections [11–13]. In addition to interacting with LPS, the TLR4/CD14 complex interacts with other exogenous and endogenous proteins, such as RSV and fibrinogen [14–16]. Interestingly, TLR4-deficient mice had delayed clearance of RSV and a Th2 response, which correlated with disease progression [17].

Considering the importance of the TLR4/CD14 complex in initiating innate immunity to RSV, we hypothesized that genetic heterogeneity in these genes might explain, at least in part, the wide spectrum of RSV bronchiolitis. Therefore, we have assessed the common TLR4 mutations and the CD14 polymorphism in infants with severe RSV bronchiolitis, versus those in infants with mild RSV bronchiolitis, by use of real-time reverse-transcriptase polymerase chain reaction (PCR) and restriction fragment–length polymorphism analysis. Our results show an association between the common TLR4 mutations, but not the CD14 polymorphism, and severe RSV bronchiolitis.

PATIENTS AND METHODS

Patients

A prospective study was conducted at the Edith Wolfson and Soroka University Medical Centers. Only previously healthy Jewish infants with confirmed RSV bronchiolitis detected by use of an EIA (TESTPACK RSV; Abbot) during November 2002 through March 2003 were sequentially recruited and included in the study, according to disease severity. Exclusion criteria included cardiac disease, chronic respiratory disease, previous wheezing episode, age >12 months, and prematurity. Group I consisted of Jewish infants hospitalized because of severe RSV bronchiolitis. The decision to hospitalize these infants was made independently by the attending physicians, on the basis of clinical findings alone, including respiratory distress requiring oxygen therapy, poor feeding with signs of dehydration, and/or apnea. The hospitalized patients were examined at admission and daily by the investigators and were assigned clinical severity scores (CSs). The CSs, as well as the oxygen saturations, were prospectively obtained as described elsewhere [18]. Group II consisted of ambulatory Jewish infants presenting with confirmed mild RSV bronchiolitis detected during the same winter period. These infants were from the same inner-city environment as were the hospitalized infants and were referred by their pediatricians. The study plan was presented prospectively to 20 primary pediatricians working in the same area. These pediatricians referred every infant suspected of having RSV bronchiolitis, irrespective of the severity of the illness, including most mildly affected but symptomatic infants. One of the investigators was always available (on call) for the referring pediatricians. The ambulatory infants were examined daily by the investigators and followed until recovery. Group III consisted of healthy adult Jewish volunteers from the same region, representing the healthy population.

Questionnaires were prospectively obtained from all hospitalized and ambulatory infants. Parents were asked for history of perinatal diseases, previous respiratory diseases, exposure to smoking, family history of asthma or allergy, number of infants in the family, exposure to pets, breast-feeding, and day care attendance.

The "Helsinki" ethics committees of both the Wolfson Medical Center and the Soroka University Medical Center approved the study, as did the National Helsinki committee for genetic research in humans and the Israeli Ministry of Health. Signed, informed consent was obtained from the parents of each infant included in the study and from the healthy adults.

Methods

DNA extraction. DNA was extracted from peripheral blood collected with EDTA by use of the Puregene DNA purification system (GENTRA), according to the manufacturer's instructions.

TLR4 genotyping. All subjects were genotyped for mutations in the *TLR4* gene (Asp299Gly and Thr399Ile) by use of an ABI PRISM 7900HT Sequence Detection System, as described elsewhere [19]. In brief, 2 primers for each mutation and an oligonucleotide probe for each allele were synthesized as follows: (1) Asp299Gly: forward, 5′-TGAAGAATTCCGATTAGCATACTTAGA-3′ and reverse, 5′-TGTGGGAAACGTTCCAAATTTACA-3′ (TaqMan probes: wild-type 5′-ACCTCGATGATATTAT-3′ and mutant 5′-ACCTCGATGGTATTAT-3′); and (2) Thr399Ile: forward, 5′-TGAGTTTCAAAGGTTGCTGTTCTC-3′ and reverse, 5′-AGGAATACTGAAAACTCACTCATTTGTT-3′ (TaqMan probes: wild-type 5′-TTAGGCTGGTTGTCC-3′ and mutant 5′-TTAGGCTGATTGTCC-3′). The wild-type *(wt)* TaqMan probes were labeled with the fluorescent dye FAM (6-carboxyfluorescein), and the probes corresponding to the mutant allele were labeled with VIC at the 5′ end. The 3′ end of the probe carried a quencher that suppressed the fluorescence of the reporter dyes. During PCR, fluorescence developed when the probe

hybridized to a perfectly matched DNA sequence, and the exonuclease activity of *Taq* polymerase separated the quencher from the reporter dye, resulting in emission of light. Amplification was performed in a final volume of 20 μL containing 5 ng of DNA, 180 pmol of each primer, 100 pmol of each probe, and 1× TaqMan Universal PCR Master Mix (Perkin-Elmer). Reactions were loaded into 96-well plates and run in an ABI PRISM 7900HT Sequence Detection System. The PCR conditions were the same as those described elsewhere [19].

CD14 genotyping. Genotyping of the CD14/−159 polymorphism was performed according to a protocol described elsewhere [20]. In brief, PCR was performed in a volume of 25 μL containing 2 mmol/L dNTP, 1.5 mmol/L MgCl, 0.625 U of *Taq* polymerase (Bioline), and 0.1 μmol/L each primer: 5′-GCCTCTGACAGTTTATGTAATC-3′ and 5′-GTGCCAACAGATGAGGTTCAC-3′. Cycling conditions were 94°C for 3 min, 28 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 30 s, and a final extension of 72°C for 6 min. PCR products were digested with 5 U of *Ava*II (New England Biolabs) for 2 h at 37°C. Products were separated on a 2.0% agarose gel.

Statistical analysis. Two major outcomes of interest were considered: (1) the frequency distribution and odds ratio (OR) of the TLR4 mutations and (2) the CD14 polymorphism in the 3 groups. Other minor outcomes were the correlation between the severity of illness indices and the frequencies of mutant alleles within group I (hospitalized infants).

Only birth weight was normally distributed and is presented as mean ± SD. The rest of the continuous variables had distributions significantly different from normal and are presented as median (minimum, maximum). Normally distributed continuous variables were examined by use of the paired or unpaired *t* test, as appropriate. The Mann-Whitney *U* test was used to compare nonnormally distributed continuous variables. Noncontinuous variables were examined by use of the χ^2 test with Monte Carlo simulation (99% confidence interval, 10,000 iterations). $P < .05$ was considered to be statistically significant.

RESULTS

Clinical characteristics. Altogether, 181 previously healthy infants infected with RSV were sequentially enrolled in the present study. Their median age was 3 months (range, 1–12 months). Ninety-nine infants with severe RSV bronchiolitis were hospitalized (group I), and 82 ambulatory infants with mild RSV bronchiolitis were followed (group II). Ninety healthy adults were recruited and represented the healthy population (group III). Twelve of the 99 hospitalized infants were hospitalized in the pediatric intensive care unit (PICU) because of severe respiratory distress, but only 1 patient required mechanical ventilation, because of apnea. The 2 groups of RSV-infected infants had similar basic clinical characteristics and variables, except for CS and oxygen saturation (table 1). The median duration of hospitalization was 3 days (range, 2–17 days). None of the hospitalized infants died. The group of infants hospitalized in the PICU differed significantly from the rest of hospitalized infants, in the following indices, which are presented as median (range): CS, 10 (8–12) vs. 8 (6–12), $P < .01$; oxygen saturation, 88% (80%–90%) vs. 93% (82%–99%), $P < .01$; and length of hospitalization, 6 (4–17) vs. 3 (2–10) days, $P < .01$. All other indices—such as age, birth weight, length of gestation, family size, days of illness before hospitalization, parents' smoking habits, pets, number of brothers, and breast-feeding—were not significantly different between the 2 groups. The healthy adult volunteer population consisted of 53 women and 37 men, with a median age of 35 years (range, 20–50 years).

TLR4 mutations. The results of analysis of TLR4 mutations in the 3 groups of subjects are shown in tables 2 and 3. The pooled TLR4 mutations Asp299Gly and Thr399Ile were

Table 1. Comparison of clinical and demographic characteristics of infants with severe and mild respiratory syncytial virus (RSV) bronchiolitis.

Characteristic	RSV bronchiolitis	
	Severe (n = 99)	Mild (n = 82)
Age, median (range), months	3.0 (1–12)	3.5 (1–11)
Female:male	43:56	36:46
Birth weight, median ± SD, g	3230 ± 533	3113 ± 485
Family size, median no. of members	4	4
Breast-feeding, no. of infants	49	33
History of asthma in first-degree family member, no. of infants	54	40
Pets at home, no. of infants	6	10
Day care, no. of infants	8	8
Family members who smoke, no. of infants	55	37
Clinical severity score, median	8.0	4.0[a]
Oxygen saturation, median, %	91.6	98[a]

[a] $P < .001$.

Table 2. Distribution of mutant Toll-like receptor 4 (TLR4) alleles among infants with severe and mild respiratory syncytial virus (RSV) bronchiolitis.

TLR4 mutation(s)	RSV bronchiolitis		OR (95% CI)	*P*
	Severe (n = 99)	Mild (n = 82)		
Pooled frequencies of Asp299Gly and Thr399Ile	20 (20.2)	4 (4.9)	4.9 (1.6–15.3)	.003
Asp299Gly	16 (16.2)	3 (3.7)	5.1 (1.4–18.1)	.014
Thr399Ile	17 (17.2)	4 (4.9)	4.0 (1.3–12.5)	.01
Cosegregation	13 (13.1)	3 (3.7)	4.0 (1.1–14.5)	.034

NOTE. Data are no. (%) of infants, unless otherwise noted. CI, confidence interval; OR, odds ratio.

significantly overrepresented among infants hospitalized with severe RSV bronchiolitis, compared with ambulatory infants with mild RSV bronchiolitis or adult control subjects (20.2% vs. 4.9% and 5.6%, respectively; $P = .003$ and $P = .004$, respectively). Furthermore, each of the TLR4 mutations was overrepresented among infants hospitalized with severe RSV bronchiolitis, compared with ambulatory infants with mild RSV bronchiolitis or adult control subjects (Asp299Gly: 16.2% vs. 3.7% and 4.4%, $P = .003$ and $P = .009$, respectively; Thr399Ile: 17.2% vs. 4.9% and 4.4%, $P = .01$ and $P = .009$, respectively). Three (25%) of 12 infants hospitalized in the PICU were carrying the mutant TLR4 alleles, compared with 17 (19.5%) of 87 infants hospitalized in the pediatric ward ($P > .05$).

We have further assessed whether demographic features may have confounded our results. Therefore, we analyzed the distribution of the demographic features of the study population, according to TLR4; we found no differences between the infants carrying the mutant TLR4 alleles and the infants carrying the *wt* TLR4 alleles, suggesting that demographic features did not bias our results (table 4). The CSs were higher and the oxygen saturation was lower in the infants carrying the mutant alleles, compared with the infants carrying the *wt* alleles, indicating that the infants carrying the mutant alleles had concrete measures of severity of illness, compared with the infants carrying the *wt* alleles. We next assessed whether the common TLR4 mutations are associated with parameters of disease severity and basic clinical characteristics and variables, among the hospitalized infants. However, among the hospitalized infants, there were no correlations between any of the severity of illness indices (CS at any day of hospitalization, saturation, or length of hospitalization) and the frequency distribution of the mutant TLR4 alleles. With regard to any of the demographic features, no differences were found between the hospitalized infants carrying the mutant TLR4 alleles and the hospitalized infants carrying the *wt* TLR4 alleles.

CD14/− 159 polymorphism. The results of the analysis of the CD14/−159 polymorphism are shown in table 5. The 3 groups did not differ significantly in the frequency of the CD14 alleles considered.

DISCUSSION

In the present study, we have shown that severe RSV bronchiolitis is associated with the common TLR4 mutations (Asp299Gly and/or Thr399Ile) but not with the CD14/−159 polymorphism. Nongenetic factors predisposing infants to severe RSV bronchiolitis could affect our results. To minimize these effects, selection of patients was performed prospectively, and the exclusion criteria were strict. Patients with prematurity, congenital heart disease, previous wheezing episodes, and chronic lung disease were not included in the present study. Other known risk factors—such as exposure to cigarette smoke, family history of atopy, and low socioeconomic status—did not differ between the hospitalized infants and the infants with mild RSV bronchiolitis. In the present report, we used a population-based association study, which may be biased as a result of population admixture or stratification. Therefore, we included only Jewish infants and Jewish adults from the same region as a second control group, excluding other ethnic groups. Though the common TLR4 mutations were overrepresented in the hospitalized infants, we found no differences, with regard to severity of illness indices, between hospitalized infants carrying the mutant alleles and those carrying the *wt* alleles. That finding, as well as those from previous publications [5–8], may indicate that other mutations or polymorphisms may account for the rest of the hospitalized infants.

The RSV genome encodes 10 proteins, 2 of which are crucial for immunity and pathogenesis: the fusion (F) protein, which is a large envelope glycoprotein, and the major surface glycoprotein (G) protein [21]. The F and G proteins were found to produce 2 distinct reactions in mice sensitized with recombinant vaccinia virus vector. Whereas the G protein induced a Th2 response characterized by eosinophilia and secretion of IL-4 and IL-5, the F protein induced a Th1 response characterized by secretion of IL-2 and interferon–γ [22, 23]. It has recently been shown that the F protein, but not the G protein, triggers the expression of proinflammatory cytokines—such as IL-12, IL-6, and tumor necrosis factor–α—by mononuclear phagocytes, in a TLR4- and a CD14-dependent manner [14]. Fur-

Table 3. Distribution of mutant Toll-like receptor 4 (TLR4) alleles among infants with severe respiratory syncytial virus (RSV) bronchiolitis and healthy adult control subjects.

TLR4 mutation(s)	Infants with severe RSV bronchiolitis (n = 99)	Control subjects (n = 90)	OR (95% CI)	P
Pooled frequencies of Asp299Gly and Thr399Ile	20 (20.2)	5 (5.6)	4.3 (1.5–12.0)	.004
Asp299Gly	16 (16.2)	4 (4.4)	4.1 (1.3–12.9)	.009
Thr399Ile	17 (17.2)	4 (4.4)	4.5 (1.4–13.8)	.009
Cosegregation	13 (13.1)	3 (3.3)	4.4 (1.2–15.9)	.018

NOTE. Data are no. (%) of subjects, unless otherwise noted. CI, confidence interval; OR, odds ratio.

thermore, Haynes et al. [17] have shown that TLR4-deficient mice (C57BL/10ScNCR) challenged with RSV exhibit impaired expression of IL-12 and IL-18 and impaired NK cell and $CD14^+$ cell pulmonary trafficking, leading to impaired virus clearance and severe RSV bronchiolitis. These findings emphasize the importance of the TLR4/CD14 complex signaling pathway in innate immunity to RSV.

Two cosegregating missense mutations in the extracellular domain of the human *TLR4* gene have recently been described. These mutations (Asp299Gly and Thr399Ile) are associated with hyporesponsiveness to inhaled LPS and an increased incidence of gram-negative sepsis [12, 13]. In a large cohort of 810 adults recruited randomly, the Asp299Gly mutation was associated with low levels of inflammatory mediators—such as IL-6, C-reactive protein, and soluble vascular cell adhesion molecule (VCAM)–1—and conferred an increased risk of severe bacterial infection [24]. Furthermore, the Asp299Gly mutation was found to be associated with a decreased risk of carotid atherosclerosis.

We have found that severe RSV bronchiolitis is significantly associated with each of the common TLR4 mutations, compared with mild RSV bronchiolitis and no infection (20.1% vs. 4.6% and 5.6%, respectively). Although the common TLR4 mutations are expressed differently in different populations [25, 26], their expression was identical in our 2 control groups and was similar to that found in a large cohort of Italians [24], suggesting that population admixture did not bias our results. That association does not necessarily mean that these mutations confer hyporesponsiveness to the RSV F protein. However, several other explanations may exist. It is possible that these mutations confer blunt immune responses to other proteins, which might be important to the immune response to RSV. That possibility may be supported by the findings of Arbour et al. [12]. According to their findings, there was no strict individual correlation between the presence of the common TLR4 mutations and hyporesponsiveness to LPS. Furthermore, monocytes bearing both TLR4 mutations were found to exhibit the same response to different purified LPS as that of the *wt* TLR4 alleles [27], suggesting that impaired recognition of other TLR4 agonists, such as heat-shock protein, were responsible for the findings of Arbour et al. Furthermore, it is possible that the common TLR4 mutations serve as markers for other mutations

Table 4. Comparison of clinical and demographic characteristics of infants carrying the common mutant Toll-like receptor 4 alleles and those carrying the wild-type *(wt)* alleles.

Characteristic	Carrier state	
	Mutant (n = 24)	*wt* (n = 157)
Age, median (range), months	2.8 (1–9)	3.5 (1–12)
Female:male	11:24	68:157
Birth weight, median ± SD, g	3209 ± 484	3172 ± 519
Family size, median no. of members	4	4
Breast-feeding, no. of infants	13	68
History of asthma in first-degree family member, no. of infants	10	85
Pets at home, no. of infants	3	12
Day care, no. of infants	2	14
Family members who smoke, no. of infants	10	82
Clinical severity score, median	8.0	7.0[a]
Oxygen saturation, median, %	92	95[a]

[a] $P < .05$.

Table 5. Distribution of the CD14/−159 polymorphic alleles among infants with severe respiratory syncytial virus (RSV) bronchiolitis, mild RSV bronchiolitis, and healthy adult control subjects.

CD14 polymorphism	Infants with RSV bronchiolitis: Severe (n = 99)	Infants with RSV bronchiolitis: Mild (n = 82)[a]	Control subjects[a]
CC	27 (27.3)	20 (24.4)	25/90 (27.8)
CT	60 (60.6)	45 (54.9)	53/90 (58.9)
TT	12 (12.1)	17 (20.7)	12/90 (13.3)

NOTE. Data are no. (%) of subjects.

[a] $P = .3$ (infants with severe RSV bronchiolitis vs. infants with mild RSV bronchiolitis).

[b] $P = .9$ (infants with severe RSV bronchiolitis vs. healthy adult control subjects).

or genetic abnormalities. However, none of the genes near the TLR4 region on chromosome 9 have yet been found to be relevant to RSV bronchiolitis; thus, there are currently no data to prove that assumption.

Baldini et al. [28] were the first to identify a C→T transition at position −159, from the major transcription site of the *CD14* gene. Interestingly, patients homozygous with the TT genotype had higher levels of soluble CD14 than those homozygous with the CC genotype. An association between the CC allele and atopic severity was recently found [20]. Considering the pathogenesis of RSV bronchiolitis that is mediated through respiratory epithelial cells [29], it seemed logical to assume that the CC allele would be associated with disease severity. Another reason to assess the association of the CD14/−159 polymorphism and RSV bronchiolitis is that epidemiological findings suggest that family history of atopy increases the risk of severe RSV bronchiolitis [30].

In the present study, no association was found between the common CD14/−159 polymorphism and disease severity. The data in the present study showing a lack of association joins other conflicting data on the role of the CD14/−159 polymorphism in the development of atopy, since no association was found in a large cohort of German children [31]. Furthermore, the TT allele, and not the CC allele, was found to be associated with atopy among the highly inbred population of the Hutterites, though only when it was part of a specific haplotype [32]. These findings raise the possibility that the CD14/−159 polymorphism may be associated with atopy only when linkage disequilibrium with other variants exists.

Our findings raise the question of the role of TLR4 mutations in the development of future asthma. In that regard, several epidemiological studies have suggested a possible association between RSV bronchiolitis and manifestation of atopy and asthma years after infection [33, 34]. It is currently accepted that both microbial and environmental stimuli diverge the infant's Th2-prone immune response to a mature Th1 immune response [35]. Therefore, mutations that confer hyporesponsiveness to microbial stimuli will theoretically impair that divergence and result in a Th2 immune response. LPS is noteworthy to mention, since it is considered to be one of the microbial stimuli that diverge the immune system into a Th1 phenotype [35]. LPS is a cell-wall component of gram-negative bacteria. Not surprisingly, high levels of exposure to LPS were inversely related to the occurrence of hay fever and atopic asthma in a large cohort of children living in rural areas of Germany, Austria, and Switzerland [36]. Since TLR4 is the principle LPS receptor, it was assumed that mutations, especially those conferring hyporesponsiveness to LPS, would be associated with hay fever and atopic asthma. However, no association was found between the 5 common TLR4 mutations and asthma or atopy, suggesting that TLR4 is not an asthma-susceptibility gene [25]. These findings should be seen in the appropriate context: LPS is not the only microbial stimulus that is recognized by the innate immunity. Other microbial stimuli, such as CpG motifs, which activate TLR9, and cell-wall components of gram-positive bacteria, such as lipoteichoic acid, which activates TLR2 [37, 38], might also be important in manipulating the divergence of the immune system. Strengthening the above are the findings that high levels of expression of CD14 and TLR2, but not TLR4, were found in children shown to be exposed to high levels of LPS [39], implying that the high levels of exposure to LPS may represent exposure to high levels of other stimuli, such as lipoteichoic acid.

In conclusion, our results demonstrate the association between the common TLR4 mutations and severe RSV bronchiolitis. Our results may emphasize the importance of genetic variability in the pathogenesis of RSV bronchiolitis. Since the activation of the TLR4/CD14 receptor complex initiates a downstream cascade of intermediate proteins, it is possible that polymorphism in these genes may harbor loss or gain of function, which may contribute to the severity of RSV bronchiolitis. Since RSV bronchiolitis is probably a multifactorial disease, more association studies are needed to clarify that issue.

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