A TLR6 polymorphism is associated with increased risk of Legionnaires’ disease

Original Article

INTRODUCTION

Legionella pneumophila (Lp), the etiologic agent of Legionnaires’ disease (LD), is an important cause of community-acquired and nosocomial pneumonia. However, the host immune and genetic determinants of human susceptibility to Lp are poorly understood. Here we show that both TLR6 and TLR1 cooperate with TLR2 to recognize Lp in transfected HEK293 cells. We also perform a human genetic association study of 14 candidate single-nucleotide polymorphisms in Toll-like receptors (TLRs) 1, 2 and 6 in 98 LD cases and 268 controls from the Netherlands. No polymorphisms in TLR1 or TLR2 were associated with LD. A TLR6 polymorphism, 359T>C (rs5743808), was associated with an elevated risk of LD in genotypic and dominant (odds ratio (OR) 5.83, \( P = 7.9 \times 10^{-5} \)) models. The increased risk in persons with 359 TC or CC genotypes was further enhanced among smokers. In a multivariate model, 359T>C was associated with a higher risk of LD (OR 4.24, \( P = 0.04 \)), than any other variable, including age and smoking. Together, these data suggest that the human TLR6 variant, 359T>C, is an independent risk factor for LD.

RESULTS

Lp is recognized by TLR1 and TLR6

To determine whether TLR2 responses to Lp are mediated through heterodimerization with TLRs 1 or 6, we measured nuclear factor (NF)-κB activity in HEK293 cells transfected with a murine TLR2 construct with or without constructs for murine TLR1 or TLR6. HEK293 cells transfected with TLR2 plus TLR1 and stimulated with \( 10^6, 10^7 \) or \( 10^8 \) colony-forming units (c.f.u.) ml\(^{-1} \) of Lp had significantly greater NF-κB activity than HEK293 cells transfected with TLR2 alone (\( P < 0.001, P < 0.005 \) and \( P < 0.005 \), respectively; Figure 1). Cells transfected with TLR2 plus TLR6 also had greater activity in response to \( 10^6, 10^7 \) or \( 10^8 \) c.f.u. ml\(^{-1} \) of Lp in comparison with cells expressing TLR2 alone (\( P < 0.01, P < 0.005 \) and \( P < 0.005 \), respectively). As a control, responses to interleukin (IL)-1β, which stimulates NF-κB activity through the IL-1 receptor, were equally robust among cells transfected with different combinations of TLR constructs or empty vector. As an additional positive control, responses to the lipopeptide Pam3Cys were present in cells transfected with TLR2 alone as well as TLR2/1 and TLR2/6 heterodimers. Responses to lipopolysaccharide, a TLR4 ligand,
Two TLR5s are summarized in Table 1. We analyzed 14 candidate polymorphisms, the clinical features of genotyped cases and controls are 

\[ P \]

Bonferroni adjustment for multiple comparisons \( (P = 0.001) \) relative to TLR2 alone; \( P < 0.005 \) relative to TLR2 alone; \( P < 0.001 \) relative to TLR2 alone.

were not detected, as expected. These results suggest that either TLR1 or TLR6 are required for full recognition of Lp by TLR2.

The nonsynonymous TLR6 polymorphism 359T>C is associated with LD. To determine whether human TLR1, 2 or 6 polymorphisms are associated with susceptibility to legionellosis, we used a case–control study of an epidemic outbreak in the Netherlands. A description of this outbreak has previously been published and the clinical features of genotyped cases and controls are summarized in Table 1. We analyzed 14 candidate polymorphisms, 5 in TLR1, 3 in TLR2 and 6 in TLR6 in 98 cases and 268 controls. Two TLR6 single-nucleotide polymorphisms (SNPs; rs3821985 and rs3775073) had Hardy–Weinberg equilibrium (HWE) \( P \)-values \( \leq 0.001 \) and were not analyzed further. As TLR1 and TLR6 are contiguous genes on chromosome 4p14, we evaluated linkage disequilibrium among five TLR6 and four TLR6 SNPs in the control population (Figure 2). The majority of \( R^2 \) values were \( < 0.65 \), indicating a low-to-moderate degree of linkage. We found no associations with LD for any of the TLR1 variants, including rs5743618, a nonsynonymous SNP at base pair 1805 in the transmembrane domain of TLR1 that regulates signaling (Table 2).26,27 Similarly, no TLR2 variant was associated with LD. The frequency of a single TLR6 SNP, rs5743808 (359T>C), a nonsynonymous variant in the extracellular leucine-rich repeat domain of the protein (encoding an isoleucine-to-threonine transition at amino-acid residue 120), was greater in cases compared with controls (genotypic analysis: \( P = 7.9 \times 10^{-5} \)); Table 2). This association remained significant after a conservative Bonferroni adjustment for multiple comparisons \( (P = 9.5 \times 10^{-4}) \). The association best fit a dominant model (comparing TT genotypes with TC/CC) with an odds ratio (OR) of 5.83 for LD \( (P = 7.9 \times 10^{-5}) \) in cases compared with controls (Table 3). Among cases, 15% carried the C allele (had TC or CC genotype) compared with 3% of controls. ORs for LD were similar whether cases were compared with all controls \( (n = 263) \) or controls without Pontiac fever \( (n = 234; \) OR of 5.83 vs 5.17, respectively), a clinical marker of Legionella exposure (Table 3).
Table 2. TLR1, TLR2 and TLR6 polymorphism genotype frequencies in Legionnaires’ disease

| SNP          | Genotype frequency (%) | Base pair (aa) | 00 | 01 | 11 | \(\chi^2\) | P-value | HWE P-value¹ |
|--------------|-------------------------|----------------|----|----|----|-----------|---------|--------------|
| TLR6         |                         |                |    |    |    |           |         |              |
| rs17616434   | Controls                |                |    |    |    |           |         |              |
| –12 422T>C   | Cases                   |                |    |    |    |           |         |              |
| rs4833095    | Controls                |                |    |    |    |           |         |              |
| + 743T>C     | Cases                   |                |    |    |    |           |         |              |
| rs3923647    | Controls                |                |    |    |    |           |         |              |
| + 914A>T     | Cases                   |                |    |    |    |           |         |              |
| rs3775073    | Controls                |                |    |    |    |           |         |              |
| + 1263A>G    | Cases                   |                |    |    |    |           |         |              |

Abbreviations: HWE, Hardy-Weinberg equilibrium; SNP, single-nucleotide polymorphism; TLR, Toll-like receptor. ¹Total numbers of cases and controls may not sum to 268 and 98, respectively, because of failed genotyping for small numbers of individuals. Controls include 29 individuals with Pontiac fever. ²00 denotes homozygosity for major (common) allele, 01 denotes hetereozygosity and 11 denotes homozygosity for minor allele in the Netherlands population. ³Base pair position given relative to ATG start site along with corresponding amino-acid (aa) within parentheses. ⁴P-value for HWE test performed in control population.

Two TLR6 SNPs (rs3821985 and rs3775073) with HWE P-values < 0.001 are presented in this table but were not analyzed further.

Table 3. Frequency of TLR6 polymorphism rs5743808 in cases and different control groups

| Genotype frequency (%) | Dominant analysis |
|------------------------|-------------------|
|                        | TT (%) | TC/CC (%) | OR (95% CI) | P-value |
| Pontiac fever included |        |          |            |         |
| Controls               | 255 (0.97) | 8 (0.03) | 0 (0.00) | 1893 | 7.9 \times 10^{-5} | 255 (0.97) | 8 (0.03) | 1.00 |
| Cases                  | 82 (0.85) | 14 (0.14) | 1 (0.01) | 5.92 | 0.04 | 82 (0.85) | 15 (0.15) | 5.83 (2.21–16.39) | 7.9 \times 10^{-5} |

Pontiac fever excluded

| Controls               | 226 (0.97) | 8 (0.03) | 0 (0.00) | 1600 | 3.4 \times 10^{-4} | 226 (0.97) | 8 (0.03) | 1.00 |
| Cases                  | 82 (0.85) | 14 (0.14) | 1 (0.01) | 5.92 | 0.04 | 82 (0.85) | 15 (0.15) | 5.17 (1.96–14.54) | 2.3 \times 10^{-4} |

Seropositive

| Controls               | 56 (1.00) | 0 (0.00) | 0 (0.00) | 9.60 | 0.002 | 56 (1.00) | 0 (0.00) | 1.00 |
| Cases                  | 82 (0.85) | 14 (0.14) | 1 (0.01) | 5.92 | 0.04 | 82 (0.85) | 15 (0.15) | 3.75 (1.12–16.08) | 0.03 |

Matched

| Controls               | 82 (0.95) | 4 (0.05) | 0 (0.00) | 1.00 |
| Cases                  | 82 (0.85) | 14 (0.14) | 1 (0.01) | 5.92 | 0.04 | 82 (0.85) | 15 (0.15) | 3.75 (1.12–16.08) | 0.03 |

Abbreviations: CI, confidence interval; Lp, Legionella pneumophila; OR, odds ratio; TLR, Toll-like receptor. ¹Controls include individuals with Pontiac fever. ²Controls exclude individuals with Pontiac fever. ³Controls with positive serology for Lp. ⁴Controls matched to cases by age, gender and place of residence within 25 km.
We then compared cases with controls with serological evidence of exposure to Lp and found that the risk of LD associated with TC/CC genotypes was further enhanced when cases were compared with seropositive controls: 15% of cases had a TC or CC genotype compared with 0% of exposed, seropositive controls and 3% of all controls (Table 3). To ensure that our results were not due to population admixture, we also examined genotype frequencies for 359T>C in 86 controls and 97 cases matched for age, sex and place of residence within 25 km. The increased risk of LD in cases as compared with matched controls was still seen, although the OR was lower than for the general control group (OR of 3.75, P = 0.03; Table 3).

We previously identified a TLR5 polymorphism (1174C>T or 392R>STOP) associated with susceptibility and two TLR4 polymorphisms (896A>G (299D>G) and 1196C>T (399T>I)) associated with resistance to LD. We found no evidence of interaction between any of these previously defined risk alleles and TLR6 359T>C (data not shown), suggesting that 359T>C is an independent susceptibility locus for LD.

TLR6 359T>C confers a higher risk of LD than other genetic and non-genetic risk factors

Previously reported risk factors for LD include older age, gender, smoking, diabetes, alcohol use and chronic respiratory illness.29–33 In our cohort, older age, diabetes mellitus and chronic respiratory disease were each significantly associated with an increased risk of LD (Table 4) in a univariate analysis. Smoking was also associated with increased LD risk that did not reach significance in the univariate analysis (P = 0.2). Conversely, alcohol use was significantly associated with protection from LD (Table 4). We performed a multivariate analysis using all variables with a P ≤ 0.2 in the univariate analysis as well as the TLR5 1174C>T and TLR4 896A>G and 1196C>T variants previously associated with LD. In the multivariate analysis, the 359T>C variant retained a significant association with LD (OR 4.24, P = 0.04, dominant analysis; Table 4).

As smoking was also associated with a greatly increased risk of LD (OR 3.51, P = 0.002) in our multivariate analysis, we stratified our analysis by smoking status. Among smokers, individuals with TC or CC genotypes had a significantly elevated risk of LD compared with controls (OR 8.75, P = 0.005, unadjusted analysis; Table 5). Among nonsmokers, TC/CC genotypes were associated with somewhat less increased risk (OR 4.07, P = 0.03, unadjusted analysis; Table 5). After adjustment for age and alcohol, both the associations among smokers and nonsmokers became nonsignificant (P = 0.11 and P = 0.06, respectively), likely due to small numbers. These results suggest that the association of 359T>C with LD may be more pronounced in smokers than nonsmokers.

We next examined NF-kB signaling in response to heat-killed Lp in HEK 293 cells transfected with constructs containing the 359T (wild type) or 359C (SNP) variant of human TLR6, but found no differences in Lp- or Pam2CSK1-mediated responses (data not shown).

DISCUSSION

Our data show that TLR6 mediates recognition of Lp and that a common polymorphism is associated with susceptibility to LD.

Table 4. Predictors of risk of Legionnaires’ disease in a Dutch population

| Characteristics | Univariate analysis | Multivariate analysis |
|-----------------|---------------------|-----------------------|
| | N | OR | 95% CI | P-value | N | OR | 95% CI | P-value |
| TLR6 359 TC/CC | 360 | 5.83 | 2.39–14.25 | <0.001 | 274 | 4.24 | 1.05–17.17 | 0.04 |
| Diabetes mellitus | 357 | 4.57 | 1.68–12.39 | 0.003 | 274 | 1.48 | 0.31–7.16 | 0.49 |
| TLR5 1174 CT/TT | 360 | 2.24 | 1.14–4.39 | 0.02 | 274 | 1.91 | 0.67–5.46 | 0.23 |
| Chronic respiratory illness | 360 | 1.54 | 1.02–2.35 | 0.04 | 274 | 0.66 | 0.17–2.51 | 0.54 |
| Smoking | 318 | 1.36 | 0.83–2.22 | 0.22 | 274 | 3.51 | 1.61–7.63 | 0.002 |
| Older age | 362 | 1.12 | 1.09–1.15 | <0.001 | 274 | 1.14 | 1.10–1.19 | <0.001 |
| Female gender | 361 | 0.82 | 0.51–1.30 | 0.39 | 274 | — | — | — |
| TLR4 1196 CT/TT | 360 | 0.40 | 0.16–0.97 | 0.04 | 274 | 0.59 | 0.16–2.11 | 0.42f |
| Alcohol use | 316 | 0.45 | 0.26–0.77 | 0.004 | 274 | 0.42 | 0.20–0.88 | 0.02 |

Abbreviations: CI, confidence interval; OR, odds ratio; TLR, Toll-like receptor. *TC and CC genotypes compared with TT genotype for TLR6 rs5743808 (359T>C) polymorphism (dominant analysis). †CT and TT genotypes compared with CC genotype for TLR4 (1174C>T). ‡Age analyzed as continuous variable. §AG and GG genotypes compared with AA genotype for TLR4 (896A>G). §CT and TT genotypes compared with CC genotype for TLR4 (1196C>T). These data pertain to combined genotype category of TLR4 896 AG/GG or TLR4 1196 CT/TT as SNPs TLR4 896A>G and 1196C>T are in complete linkage disequilibrium in this population (all 896AA genotypes co-segregate with 1196CC and all 896AG genotypes co-segregate with 1196 CT).

Table 5. Frequency of TLR6 polymorphism rs5743808 by smoking status

| Characteristic | Genotype frequency (%) | Dominant analysis |
|---------------|------------------------|------------------|
| | TT | TC | CC | χ² | P-value | TT (%) | TC/CC (%) | OR (95% CI) | P-value |
| Smokers | | | | | | | | |
| Controls | 90 (0.98) | 2 (0.02) | 0 (0.00) | | | 90 (0.98) | 2 (0.02) | 1.00 |
| Cases | 36 (0.84) | 6 (0.14) | 1 (0.02) | 9.63 | 0.005 | 36 (0.84) | 7 (0.16) | 8.75 (1.54–88.61) | 0.005 |
| Nonsmokers | | | | | | | | |
| Controls | 129 (0.96) | 5 (0.04) | 0 (0.00) | | | 129 (0.96) | 5 (0.04) | 1.00 |
| Cases | 38 (0.86) | 6 (0.14) | 0 (0.00) | 5.61 | 0.03 | 38 (0.86) | 6 (0.14) | 4.07 (0.97–17.70) | 0.03 |

Abbreviations: CI, confidence interval; OR, odds ratio; TLR, Toll-like receptor.
In vitro, the presence of TLR1 or TLR6 was required for maximal TLR2-mediated responses to heat-killed Lp in transfected HEK cells. In vivo, a natural variant of human TLR6, 359T>C, was associated with elevated risk of LD. Previously published work has implicated TLR2 in the mammalian innate immune response to Legionella. Although the majority of Gram-negative organisms have an lipopolysaccharide that signals through TLR4, the lipopolysaccharide structure of Legionella is atypical and appears to predominantly signal through TLR2. In addition, the Legionella structural protein, peptidoglycan-associated lipoprotein, signals via TLR2. Studies of Legionella pneumonia infection in TLR2−/− mice have shown 10- to 100-fold higher c.f.u. in the lung compared with wild-type counterparts and enhanced intracellular growth of Legionella has been shown in TLR2-deficient murine macrophages. Our functional data suggest that TLR2/6 and TLR2/1 cooperate to recognize Lp. Our genetic association results further suggest that TLR6 is a genetic locus of LD susceptibility. Although we did not detect an association between TLR2 polymorphisms and LD, these results do not rule out a contribution of TLR2 variants to LD susceptibility because only three candidate SNPs in TLR2 (597T>C, 1350T>C and 2258G>A) were evaluated in our study. Our study has several potential limitations. Although our results suggest an association of TLR6 with LD, we cannot exclude the possibility that the 359T>C SNP is in linkage with a nearby causative variant and serves as a marker for this alternative risk locus. This is in continued with the link between TLR6 54-kb segment of chromosome 4p14 and the potential role of TLR10 variants in LD susceptibility was not evaluated in this study. The similar NF-kB responses of the TLR6 359T and 359C variants in response to heat-killed Lp could be explained if the 359T>C polymorphism primarily modulates initial uptake or intracellular trafficking of live bacteria by the macrophage rather than NF-kB- driven cytokine responses in response to heat-killed Lp. However, we have not evaluated this possibility. As with other genetic association studies, genotyping error can occur or confounding may exist because of the unaccounted-for effects of population stratification or ethnic admixture. We judged ethnic admixture to be unlikely to cause confounding, as >95% of both cases and controls were of Caucasian Dutch background. We also included place of residence (along with age and sex) as one of the matching criteria in the original study design to control for possible population stratification and found that the association of the 359T>C SNP with LD was similar whether we used the general control group (OR 5.83, P = 7.9 × 10−5) or the smaller, matched control (OR 3.75, P = 0.03) group as the comparator. Another potential weakness of this study is the relatively small number of LD cases (98 analyzed out of 188 identified in the original outbreak). Nonetheless, our case sample size is larger than most outbreaks reported in the medical literature. Ideally, these findings will be investigated in a future cohort of different ethnicity.

Few genetic association studies have addressed the role of TLR6 in infectious diseases. One study reported an association of 359T>C in cases of 749C>T, among other polymorphisms, with tuberculosis in an African population. The TLR6 variant 749C>T has a reported association with increased risk of invasive aspergillosis after stem cell transplantation. The mechanisms responsible for the association of TLR6 variants with these diverse pathogens—from an intracellular bacterium to an extracellular mould—remain unknown. Several studies have investigated the role of TLR6 variants in mediating altered cytokine signaling in response to pathogens or pathogen motifs. In functional studies, 745T (249S) and the synonymous SNP 1083C have been reported to be associated with decreased whole blood IL-6 responses to bacterial lipopolysacids, and SNP 1083C was additionally associated with decreased IL-6 responses to Mycobacterium tuberculosis lysate and Bacillus Calmette Guérin. Other authors have demonstrated a link between TLR6, phagocytosis and autophagy. For example, TLR6 and TLR2 cooperate to recognize zymosan, a TLR2 ligand from yeast, and recruit the autophagy marker, LC3, to zymosan-containing phagosomes. The magnitude of LD risk posed by TLR6 359T>C variant (OR 5.83, dominant analysis) is greater than that posed by other polymorphisms previously associated with LD, specifically, TLR5 1174C>T, TLR4 896A>G and TLR4 1196C>T (ORs 0.40–2.24; Table 4). Furthermore, we found no evidence of an interaction between the TLR6 359C allele and any of the TLR5 or TLR4 risk alleles. These results support a role for human TLR6 in the immunopathogenesis of LD. If validated in other genetic association studies of LD, one could imagine the usefulness of this SNP in outbreeds settings, where individuals at highest risk could be targeted for pre-emptive therapy. Similarly, the TLR6 359T>C polymorphism could be used prospectively to identify immunocompromised individuals with enhanced genetic risk for legionellosis.

MATERIALS AND METHODS

Reagents, bacteria and cells

Ultrapure lipopolysaccharide was from Escherichia coli 0111:B4 (InvivoGen, San Diego, CA, USA). Lipopolysaccharide Pam3Cys-SKKKK (tri-acylated, Pam3) was from InvivoGen. IL-1β was from R&D Systems Minneapolis, MN, USA, and LPS Philadelphia serogroup 1 strain (ATCC, Manassas, VA, USA, 33152) was heat killed at 65 °C for 30 min at concentrations of 107, 106 or 105 c.f.u. ml−1 (corresponding to multiplicity of infections of ~2.5:1, ~25:1 and ~250:1, respectively) for stimulation assays. HEK293 cell line (obtained from A Hajar, Seattle, WA, USA) were cultured in a 96 well flat-bottomed tissue culture plate at ~5 × 104 cells per well in Dulbecco’s modified Eagle’s medium (Mediatech, Manassas, VA, USA) plus 10% heat-inactivated fetal bovine serum (HyClone, Logan, UT, USA). Cells were transiently transfected with 5 µl of transfection reagent comprised of a 1:1 mix of 0.25 µl CaCl2 containing 2 × BES-buffered solution (50 mM N,N-bis-(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES), 280 µM NaCl and 1.5 mM NaH2PO4) and DNA expression vectors for Renilla luciferase (driven by constitutively active β-actin promoter (control for transfection efficiency)), endothelial leukocyte adhesion molecule-1 firefly luciferase reporter plasmid (driven by NF-kB, murine MD2 and murine CD14, along with the following HA-tagged constructs: murine TLR2 alone, murine TLR2 with murine TLR6 or murine TLR2 with murine TLR1). The total amount of DNA added per well was adjusted to 0.05 µg by the addition of empty vector transfected cells. Transfected cells were washed once after 4 h and stimulated the following day with Lp lysates, or heat-killed Lp for 4 h, then lysed and processed for luciferase readings per the manufacturer’s instructions for the Dual Luciferase Reporter Assay System (Promega, Madison, WI, USA).

Human subjects and data collection

Approval for human study protocols was obtained from the human subjects review boards at the University of Amsterdam Medical Center and the University of Washington. All procedures for human subjects were consistent with ethical standards set by the 1964 Helsinki Declaration. Each participant gave written informed consent. Enrollment of cases and controls from an LD outbreak in Bovenkarspel, has been described previously. Of the 188 cases (133 confirmed and 55 probable) included in the original investigation of the outbreak, DNA and epidemiologic data were available from 98 cases (84 confirmed LD and 14 probable LD) and 268 controls (Table 1) for this study. A confirmed case of LD was defined as radiographic finding of pneumonia and laboratory evidence (positive urine antigen, sputum culture or serology) of Lp infection. A probable case was defined as a radiographic finding of pneumonia in a person attending the flower show during the epidemic period and no evidence of alternate pathogens. Individuals recruited as controls were exhibitioners who worked at the flower show, completed a questionnaire and had blood drawn once after 4 h and stimulated the following day with Lp ligands, or heat-killed Lp for 4 h, then lysed and processed for luciferase readings per the manufacturer’s instructions for the Dual Luciferase Reporter Assay System (Promega, Madison, WI, USA).
Genomic techniques

Genomic DNA was purified from peripheral blood leukocytes from 10 ml of blood. Genotyping was performed using a chip-based matrix-assisted laser desorption/ionization time-of-flight Mass ARRAY technique (Sequenom, San Diego, CA, USA), as described previously.46 Cluster plots were visually inspected to ensure accurate genotyping.

Statistics

Fourteen candidate polymorphisms, 5 in TLR1, 3 in TLR2 and 6 in TLR6, were genotyped in cases and controls. We examined HWE P-values and SNP genotypic frequencies in the cases and controls using Stata 11.1 software (StataCorp, College Station, TX, USA) and the user-written package ‘GENASS’.47 All SNPs analyzed for association with LD were in HWE using a cutoff P-value of ≥0.001 (χ²-goodness-of-fit test) in the control group to ensure that there were no genotyping errors or major effects of population heterogeneity. Two SNPs in TLR6 (rs3821985 and rs3775073) showed significant departure from HWE among control subjects (P<0.001) and were not further evaluated. The remaining 12 SNPs passed the HWE P-value test (P>0.001) and were assessed for association with LD, using a genotypic model in the first-pass analysis; those that had a significant association (P<0.05) were then investigated under dominant and recessive genetic models. In the dominant model, carriers of the less common allele (01 and 11 genotypes) were compared with homozygous subjects for the major allele (00 genotype). In the recessive model, individuals homozygous for the rare allele (11 genotypes) were compared with heterozygotes and major allele homozygotes (01 and 11 genotypes). SNP associations with LD were also analyzed within subgroups of smokers and nonsmokers and in cases compared with specific control groups (controls without Pontiac fever, seropositive controls or matched controls). For the TLR6 359T>C polymorphism, we used univariate logistic regression to assess the relative magnitude of risk conferred by this genetic variant compared with traditional risk factors for Legionella pneumophila. Induction of direct antimicrobial activity through mammalian Toll-like receptor receptors. Science 2001; 291: 1544–1547.

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