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ORIGINAL ARTICLE

Expression Profiles of Toll-Like Receptors in the Differentiation of an Infection with *Borrelia burgdorferi* Sensu Lato Spirochetes

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Abstract The similarity of Lyme borreliosis to other diseases and its complex pathogenesis present diagnostic and therapeutic difficulties. The changes that occur at the cellular and molecular levels after a *Borrelia* sp. infection still remain poorly understood. Therefore, the present study focused on the expression of *TLR* and *TLR*-signaling genes in human dermal fibroblasts in the differentiation of an infection with *Borrelia burgdorferi* sensu lato spirochetes. Normal human dermal fibroblasts were cultured with the spirochetes of *Borrelia burgdorferi* sensu stricto, *Borrelia afzelii* and *Borrelia garinii*. Total RNA was extracted from the cells using TRIzol reagent. The analysis of the expression profiles of *TLRs* and *TLR*-related genes was performed using commercially available oligonucleotide microarrays of HG-U133A. The GeneSpring 12.0 platform and significance analysis of microarrays were used for the statistical analysis of microarray data. The analyses using the oligonucleotide microarray and QRT-PCR techniques permitted to identify the genes encoding *TLR4* and *TLR6* as specific for infection with *B. afzelii* and *B. burgdorferi* sensu stricto. In turn, *TLR3* was only characteristic for an infection with *B. burgdorferi* sensu stricto. There were no changes in the *TLR* gene expression after infection with *B. garinii*. Our findings confirm that *Borrelia* has a major effect on fibroblast gene expression. Further characterization of changes in gene expression may lead to valuable insights into the role of the toll-like receptor in the pathogenesis of Lyme disease and may provide guidelines for the development of diagnostic markers for an infection with a particular *Borrelia* genospecies. Moreover, this will help to identify better treatment strategies for Lyme disease.

Keywords Toll-like receptors · *Borrelia burgdorferi* sensu lato spirochetes · Fibroblasts cultures · Oligonucleotide microarray · QRT-PCR

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Introduction

Lyme borreliosis (Lyme disease) is a multisystem infectious disease caused by the *Borrelia burgdorferi* sensu lato spirochetes, which are transmitted to humans through the bite of infected ticks. This is one of the most common tick-borne diseases in Europe, Asia and the United States, and the incidence of this disorder is independent of age and gender. The similarity to other diseases and the complex pathogenesis present diagnostic and therapeutic difficulties (Biesiada et al. 2012; Gęca et al. 2012; Gola et al. 2014; Stanek and Reiter 2011).

To date, 15 genospecies of Borrelia spirochetes are known, of which only seven are pathogenic for humans: *Borrelia burgdorferi* sensu stricto, *Borrelia afzelii*, *Borrelia garinii*, *Borrelia bissetti*, *Borrelia spielmanii*, *Borrelia lusitaniae* and *Borrelia valaisiana*. The clinical signs of this disease correlate with the spirochete genospecies. Lyme arthritis is a common late manifestation of the late antibiotic treatment of a *B. burgdorferi* sensu stricto infection, while from an epidemiological point of view *B. garinii* is the most common agent in neuroborreliosis and *B. afzelii* is related to late skin lesions (Biesiada et al. 2012; Gęca et al. 2012; Stanek and Reiter 2011).

Borrelia spirochetes express a wide variety of lipoproteins on their surface, which play an essential role in pathogenesis, in particular, the family of Osp proteins. It has also been shown that the stimulation of human cells by a *Borrelia* infection induces the development of inflammation through the activation of Toll-like receptors 1, 2, 4, 5 and 6 (TLR1, TLR2, TLR4, TLR5 and TLR6). TLRs are involved in an innate immune response and may play a key role in the clinical diagnosis of Lyme disease (Bernardino et al. 2008; Cervantes et al. 2014; Takeda and Akira 2005).

TLRs, which have the unique ability to bind to specific fragments of pathogens, are a transmembrane receptor group. They recognize highly conserved structural motifs known as pathogen-associated molecular patterns, which are exclusively expressed by various microorganisms or danger-associated molecular patterns, which are endogenous molecules that are released from necrotic or dying cells. TLR signaling is divided into two distinct molecular pathways—a MyD88-dependent pathway that leads to the production of inflammatory cytokines and a MyD88-independent pathway that is associated with the stimulation of interferon-β (Takeda and Akira 2005).

Recent studies have shown that *TLR* and *TLR*-signaling genes can play an important role in infections that are caused by different types of *Borrelia* spirochetes (Lieskovska and Kopecky 2012; Oosting et al. 2011; Strle et al. 2012). However, changes at the cellular and molecular level after a *Borrelia* sp. infection remain still poorly understood.

Therefore, the present study focused on the expression of *TLR* and *TLR*-signaling genes in normal human dermal fibroblasts in the differentiation of an infection with three major Lyme *Borrelia* genospecies.

Materials and Methods

Materials

Normal human dermal fibroblasts (NHDF cell line) were obtained from Clonetics (CC-2511; San Diego, CA, USA) (Alikhani et al. 2007; Roomi et al. 2009). The reference strains of *Borrelia* spirochetes, including *B. burgdorferi* sensu stricto (B31, ATCC 35210), *B. afzelii* (VS 461, ATCC 51567) and *B. garinii* (20047, ATCC 51383) (Baranton et al. 1992), were purchased from ATCC (Manassas, VA, USA) and derived from the National Institute of Public Health in Warsaw (Poland). The research was conducted using a commercially available cell line of human dermal fibroblasts and reference strains of *Borrelia* and, therefore, approval of the ethics committee was not required.

Methods

Cell Culture Conditions

The bacterial culture was performed in a BSK-H medium at 37 °C in microaerophilic conditions. The microscopic analysis of the culture was performed after 7 days of growth. The number of cells was monitored by counting the cells in a Bürker chamber.

Normal human dermal fibroblasts were routinely maintained in an fibroblast basal medium (FBM medium; Lonza, Basel, Switzerland), which was supplemented with human fibroblast growth factor-basic, insulin and gentamicin (FGM™ SingleQuots™; Lonza, Basel, Switzerland) at 37 °C in a 5 % CO_2 incubator (Direct Heat CO_2; Thermo Scientific, Waltham, MA, USA). Both cell number and viability were monitored by counting the cells in the Bürker chamber after staining them with 0.2 % trypan blue (Biological Industries, Beit HaEmek, Israel). The experiment was performed on cells in the logarithmic phase of growth under conditions of ≥98 % viability as assessed by trypan blue exclusion.

For the experiment, NHDF cells were washed with phosphate-buffered saline (Lonza, Basel, Switzerland) and seeded into culture dishes (Nunc, Wiesbaden, Germany). After reaching subconfluence, the suspensions of three genospecies of *Borrelia* spirochetes in the BSK-H medium were added to the NHDF cells maintained in an FGM medium at a ratio of 10:1 bacteria to fibroblasts. The co-culture medium was a mixture of 10 % BSK-H and 90 % FGM medium. The study and control fibroblasts were harvested after 24 h for further analysis. The cells were pelleted and frozen at −70 °C for 24 h until the extraction of the nucleic acids.

Ribonucleic Acid Extraction

Total RNA was extracted from the cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The total RNA concentration

was determined by a spectrophotometric measurement using a Gene Quant II RNA/DNA Calculator.

Oligonucleotide Microarray Analysis

An oligonucleotide microarray analysis was performed on the control NHDF cells, samples of *B. afzelii*-, samples of *B. garinii*- and samples of *B. burgdorferi* sensu stricto-infected NHDF cells.

The analysis of the expression profile of the *TLR* and *TLR*-signaling genes was performed using commercially available oligonucleotide microarrays HG-U133A (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's recommendations. Each gene chip contains 22,238 probe sets that correspond to more than 18,400 transcripts and 14,500 well-characterized human genes. Raw data were deposited in the Gene Expression Omnibus database (accession no. GSE68741).

Quantitative RT-PCR Assay

Detection of the expression of *TLR1, TLR2, TLR3, TLR4, TLR5, TLR6* and glyceraldehyde-3-phosphate (GAPDH) mRNAs was performed using a quantitative real-time (RT) PCR with SYBR Green chemistry (SYBR Green QuantiTect Probe RT-PCR Kit, Qiagen, Valencia, CA, USA) according to the manufacturer's instructions.

The point at which a PCR product is first detected above a fixed threshold, which is termed a cycle threshold (*C*t), was determined for each sample. To quantify the results obtained by RT-PCR, a standard curve method, which was described by Berezowski et al. (2012), was used. The obtained results were recalculated per 1 μg of total RNA.

Statistical Analysis

Microarray data analysis was performed using the GeneSpring 12.0 platform (Agilent Technologies UK Limited, South Queensferry, UK) and significance analysis of microarrays (SAM). Fluorescence intensity values of all 22,238 mRNA transcripts for HG-U133A chips were simultaneously normalized using the robust multiarray average algorithm. Sample quality was assessed by performing 3D principal component analysis (PCA), an analysis of the normalized fluorescence signal values for the hybridization control probes and the 3′/5′ ratios for the internal controls. For further study, we selected eight transcripts of the *TLR* genes (the oligonucleotide microarray HG-U133A contained probes for *TLR1–8*) and 867 transcripts of the TLR-signaling genes from the NetAffx Analysis Center database of Affymerix (http://www.affymetrix.com/analysis/index.affx). The normalized microarray data were used to compile a list of the selected *TLR* and *TLR*-signaling genes whose expression appeared to be up- or down-regulated by a cutoff of at least a 1.1-fold change. The unpaired *t* test was applied to detect differentially expressed genes at $p < 0.05$ when the analysis was performed by GeneSpring 12.0. In the SAM analysis to identify significantly differentially expressed genes, score and *q* value parameters were used (Chrominski and Tkacz 2015).

A Venn diagram was used to show the logical relationships between the groups of differentially expressed genes. Gene ontology analysis was carried out with the PANTHER 8.0 Classification System database (protein analysis through evolutionary relationships; http://www.pantherdb.org) to classify the genes based on their biological process.

Statistical analysis of the QRT-PCR results was performed using Statistica 10.0 software. Nonparametric tests were used for the statistical analyses—the Kruskal–Wallis test and post hoc multiple test.

Results

The analyses were repeated three times in each sub-group. It was found that all of the microarray samples passed the quality criteria based on the quality control results (PCA, hybridization controls and internal controls) and were then classified into four groups and used for further analyses (data not shown).

Differential Expression of TLR-Like Receptor Genes

The expression of *TLR* genes was compared between the *B. afzelii*-infected NHDF and the control cells, the *B. garinii*-infected NHDF and the control cells and the *B. burgdorferi* sensu stricto-infected NHDF and the control cells. Of the eight selected *TLR* transcripts, three expressed more than 1.1-FC in the *Borrelia*-infected cells vs. the control cells at $p < 0.05$ (*t* test) (Table 1).

Among these genes, *TLR3* and *TLR4* were up-regulated, whereas *TLR6* was down-regulated. The analysis allowed the genes encoding *TLR4* and *TLR6* as specific for an infection with *B. afzelii* and *B. burgdorferi* sensu stricto to be selected. Additionally, *TLR3* was the only characteristic of an infection with *B. burgdorferi* sensu stricto.

Validation of TLR Microarray Data by QRT-PCR

The expression levels of *TLR3, TLR4* and *TLR6* that had the highest fold change in the microarray analysis were analyzed using QRT-PCR to validate the up-regulation or down-regulation that was found in the arrays.

Table 1 Characteristics of TLR genes which exhibit differential expression in the *Borrelia*-infected NHDF cells vs. control

Probe set	Gene symbol	Fold change		
		B. afzelii	*B. garinii*	*B. burgdorferi* sensu stricto
210176_at	*TLR1*	1.03↑	1.01↑	1.06↑
204924_at	*TLR2*	1.07↑	1.04↑	1.03↓
206271_at	*TLR3*	1.08↓	1.07↓	1.12↑*
221060_s_at	*TLR4*	1.18↑*	1.06↑	1.22↑*
210166_at	*TLR5*	1.05↓	1.09↑	1.03↑
207446_at	*TLR6*	1.20↓*	1.09↑	1.25↓*
207446_at	*TLR7*	1.07↓	1.04↑	1.00↑
220832_at	*TLR8*	1.02↓	1.01↓	1.04↑

Statistical significance: * $p < 0.05$, *t* test; ↑, ↓ higher and lower expression in the *Borrelia*-infected NHDF cells vs. control

Differential Expression of the TLR-Signaling Genes

The expression of TLR-signaling genes was compared between fibroblasts that had been infected with the three *Borrelia* genospecies versus the control cells.

In the first step, the results that had been obtained through the oligonucleotide microarray technique were analyzed using the GeneSpring 12.0 platform. In the cell culture that had been infected with *B. afzelii*, 155 genes demonstrated significant differences of FC >1.1 compared with the control. In the cell culture that had been infected with *B. garinii*, 125 genes demonstrated significant differences of FC >1.1 compared with the control. In turn, in the cell culture that had been infected with *B. burgdorferi* sensu stricto, 171 genes demonstrated significant differences of FC >1.1 compared with the control.

The comparison between fibroblasts that had been infected with *B. afzelii* and the control cells demonstrated significant differences at *q* value <5 and score >3 in the expression of 17 transcripts. In the cell culture that had been infected with *B. garinii*, 12 genes demonstrated significant differences of FC >1.1 at *q* value <5 and score >3 compared with the control. The comparison between the fibroblasts that had been infected with *B. burgdorferi* sensu stricto and the control samples demonstrated significant differences of FC >1.1 at *q* value <5 and score >3 in the expression of seven transcripts.

The Venn diagram (Fig. 1) shows that 48 genes for the infection with *B. afzelii*, 17 genes for the infection with *B. garinii* and 56 genes for the infection with *B. burgdorferi* sensu stricto achieved the criteria of GeneSpring and SAM for typing the genes with significant differences in transcriptional activity simultaneously. These genes were selected for further analysis to differentiate the infections with particular types of *Borrelia* spirochetes.

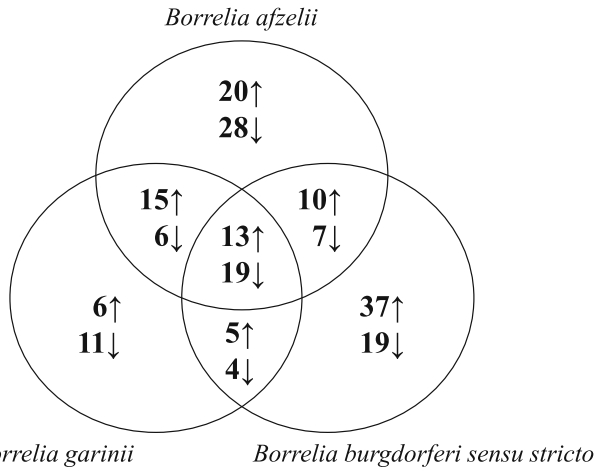

Fig. 1 Venn diagram showing the number of genes up- (*uparrows*) and down-regulated (*downarrows*) at different combinations of infection with the spirochete genospecies vs. the control

Functional Classification of the *TLR*-Signaling Genes that were Altered by *Borrelia* Spirochetes

In the final stage of the analysis, the strength of the differentiating *TLR*-related genes in differentiating between a *B. afzelii, B. garinii* and *B. burgdorferi* sensu stricto infection was estimated based on the results of an over-representation test. To classify the genes into biological categories, the Gene Ontology annotations of the differentially expressed *TLR*-signaling genes in the fibroblasts that had been stimulated with *B. afzelii*, *B. garinii* and *B. burgdorferi* sensu stricto were analyzed. PANTHER found several functional categories that were significantly enriched in this gene set compared to the entire NCBI reference list of the human genome. Categories that showed a $p < 0.05$, as determined by the binomial statistics, were considered to be potentially interesting.

Analysis indicated that among the 48 differentially expressed genes in the *B. afzelii*-infected cells, only ten that could be classified in two biological processes were significant. Among the 17 differentially expressed genes in the *B. garinii*-infected cells, 14 that could be classified in nine biological processes were significant, whereas among the 56 differentially expressed genes in the *B. burgdorferi* sensu stricto-infected cells, 22 that could be classified in eight biological processes were significant. A comparison of gene expression related to the TLRs in the NHDF cells that had been infected with different *Borrelia* genospecies also demonstrated significant common *TLR*-related genes for an infection with two genospecies (*B. afzelii* and *B. garinii*, *B. afzelii* and *B. burgdorferi* sensu stricto, *B. burgdorferi* sensu stricto and *B. garinii*) and with all three of the genospecies studied (*B. burgdorferi* sensu lato) (Table 2).

Discussion

Our attention focused on the influence of *Borrelia* spirochetes on the expression of the TLR and *TLR*-related genes to aid in the development of diagnostic markers and targets for a therapeutic intervention in Lyme disease. The immune response of the skin is essential in controlling the development of the Lyme borreliosis (Salazar et al. 2009; Schramm et al. 2012) and, therefore, we used normal dermal human fibroblasts in our study. They are considered to be part of the immune system due to their highly specialized roles in tissue homeostasis, leukocyte recruitment and the regulation of inflammation (Marchal et al. 2009). Only a few studies have evaluated the underlying molecular mechanism that triggers its activation in *Borrelia*-induced cells (Elkon et al. 2007).

When we were launching our study, we found a few published reports on gene expression in human dermal fibroblasts that had been infected with *Borrelia* in the differentiation of infections with various genospecies of spirochetes (Schramm et al. 2012). Analyses using the oligonucleotide microarray and QRT-PCR techniques allowed the genes encoding *TLR4* specific for infection with *B. afzelii* and *B. burgdorferi* sensu stricto to be selected. Lipopolysaccharide (LPS) is a ligand for TLR4 whose expression significantly increased in the culture of NHDF cells that had been infected with the spirochetes of *B. afzelii* and *B. burgdorferi* sensu stricto. Despite the fact that *Borrelia burgdorferi* does not have LPS in the cell membrane, the activation of some TLRs after a spirochete infection can cause the overexpression of *TLR4*. Similar to our results, the study of Bernardino et al. (2008) showed an increased expression of *TLR4* in brain tissues after a *B. burgdorferi* sensu stricto infection. In turn, *TLR3* was only characteristic for an infection with *B. burgdorferi* sensu stricto, which is in agreement with the findings of Elkon et al. (2007). We also observed that the expression change of *TLR6* was specific for infections with *B. afzelii* and *B. burgdorferi* sensu stricto.

Moreover, genes that characterized a statistically significant change expression only after infection with particular *Borrelia* genospecies were also observed among the *TLR*-signaling genes. As a result of infection of fibroblast culture with a specific *Borrelia* genospecies, variable expression of *TLR*-associated genes was observed, specific for one genospecies only. *B. afzelii* and *B. burgdorferi* sensu stricto—genes that are mainly involved in immune system processes and *B. garinii*—genes that are mainly involved in apoptosis and biological regulation. These findings may have an essential role in the early diagnostics of Lyme disease. Conversely, Schramm et al. (2012) did not identify any specific relevant strain-related transcriptional pathway after infections with different strains of *B. burgdorferi* sensu stricto. They indicated that the three strains of *B. burgdorferi* sensu stricto, which had been isolated from different environments and stages of Lyme disease, exhibited a similar profile of inflammation that included a strong induction of chemokines (CXCL1 and IL-8) and IL-6 cytokine, which are mainly involved in the chemoattraction of immune cells. Jones et al. (1994) revealed that *B. burgdorferi* increased IL6 production in fibroblasts and that this increase correlated with the pathogenicity of Lyme disease. Our study also confirmed the overexpression of *IL6* in cultures that had been infected with *B. burgdorferi* sensu stricto. In turn, the enhanced production of IL-6 may be involved in the activation of B cells, which produce immunoglobulins. Moreover, previous studies have shown that *Borrelia* surface lipoproteins are present extracellularly as a multiprotein complex. This complex combines with immunoglobulins to protect the surface of spirochetes and to reduce their availability. This phenomenon can affect the duration of an infection (Dorward et al. 1992; Jabłońska et al. 2003).

Our research indicated that the *APP* gene (amyloid β precursor protein) was also up-regulated after both *B. burgdorferi* sensu stricto and *B. garinii* infections. The protein encoded by this gene is the source of β-amyloid peptides, which are recognized as key components in the pathophysiology of Alzheimer's disease. Similarly, Miklossy (2008) and Miklossy et al. (2004, 2006) and Carter (2011) observed an analogy between Alzheimer's disease and *B. burgdorferi* sensu stricto and *B. garinii* infections.

Our results revealed a change in the *TLR* and *TLR*-related gene expression, which are involved in the activation of the NF-κB pathway, which produces inflammatory molecules including chemokines, cytokines and antimicrobial peptides, in the cells that had been cultured with

Table 2 Characteristics of genes related with TLRs which exhibit differential expression in the *Borrelia*-infected NHDF cells

Biological process	*p* value	Genes
B. afzelii		
Immune system process	0.009	*MAPK14*↑, *OTUB2*↓, *IKBKB*↑, *RBCK1*↓, *CCL2*↓, *FMOD*↑, *CLEC2B*↓, *SVEP1*↑
Protein phosphorylation	0.010	*MAPK14*↑, *IKBKB*↑, *RIPK1*↑, *CDKL2*↓, *FMOD*↑
B. garinii		
Biological regulation	0.008	*ARAP2*↓, *NFKB2*↑, *SERPINB13*↑, *BATF3*↓, *NRIP2*↑, *IFI16*↑, *TNFRSF25*↑, *IL6*↓
Cell communication	0.007	*ARAP2*↓, *NFKB2*↑, *KHRSP*↑, *TNFRSF25*↑, *TNFAIP6*↑, *IL6*↓, *SPHK1*↓
Induction of apoptosis	0.005	*KHRSP*↑, *TNFRSF25*↑
Negative regulation of apoptosis	0.003	*IL6*↓, *TNFRSF25*↑
Nucleobase-containing compound metabolic process	0.003	*NFKB2*↑, *LRRFIP2*↓, *BATF3*↓, *NRIP2*↑, *IFI16*↑, *KHRSP*↑, *CREG1*↑, *CNOT8*↑
Regulation of biological process	0.005	*NFKB2*↑, *SERPINB13*↑, *BATF3*↓, *NRIP2*↑, *IFI16*↑, *TNFRSF25*↑, *IL6*↓
RNA metabolic process	0.002	*NFKB2*↑, *LRRFIP2*↓, *BATF3*↓, *NRIP2*↑, *IFI16*↑, *KHRSP*↑, *CNOT8*↑
Transcription from RNA polymerase II promoter	0.003	*NFKB2*↑, *LRRFIP2*↓, *BATF3*↓, *NRIP2*↑, *IFI16*↑, *KHRSP*↑
Transcription DNA-dependent	0.009	*NFKB2*↑, *LRRFIP2*↓, *BATF3*↓, *NRIP2*↑, *IFI16*↑, *KHRSP*↑
B. burgdorferi sensu stricto		
B cell-mediated immunity	0.008	*SIGLEC9*↓, *IL27RA*↓, *SIGLEC8*↓
Blood circulation	0.010	*NOS2*↓, *NOS3*↓, *ADRB2*↑
Cell communication	0.001	*CXCL10*↑, *NOS2*↓, *TSPAN6*↑, *ADAMTS13*↓, *AGER*↓, *AR*↑, *NOS3*↓, *GPR132*↓, *AZI2*↑, *IL27RA*↓, *MAPKAPK3*↓, *CARD10*↓, *VNN1*↓, *ADRB2*↑, *LTB4R2*↓, *SIRPB1*↑, *STXBP2*↓
Immune response	0.001	*OASL*↓, *IL4R*↓, *SIGLEC9*↓, *IL27RA*↓, *ADRB2*↑, *SIGLEC8*↓
Immune system process	0.005	*CXCL10*↑, *TSPAN6*↑, *OASL*↓, *IL4R*↓, *SIGLEC9*↓, *IL27RA*↓, *ADRB2*↑, *NLRP3*↓, *SIGLEC8*↓
Lysosomal transport	0.007	*ADRB2*↑, *STXBP2*↓
Response to biotic stimulus	0.006	*CXCL10*↑, *NOS2*↓
Response to stimulus	0.007	*CXCL10*↑, *NOS2*↓, *TSPAN6*↑, *OASL*↓, *IL4R*↓, *NOS3*↓, *SIGLEC9*↓, *IL27RA*↓, *MAPKAPK3*↓, *ADRB2*↑, *NLRP3*↓
B. burgdorferi sensu lato		
Biological adhesion	0.010	*FN1, ICAM1, ANGPTL2, KIAA0247*
Cell adhesion	0.006	*FN1, CDK10, TCF7, IFNGR1, CMKLR1, IL8, ANGPTL2, KIAA0247, IL11RA*
Cell–matrix adhesion	0.007	*FN1, ANGPTL2*
Response to stimulus	0.008	*ICAM1, TCF7, IRF2, C3, IFNGR1, IL8, KIAA0247, IL11RA*
B. afzelii and *B. garinii*		
RNA splicing	0.005	*KHRSP*↑↑, *QKI*↑↑
B. afzelii and *B. burgdorferi* sensu stricto		
Hemopoiesis	0.004	*IFNA17*↑↑
Immune response	0.001	*IFNA17*↑↑, *OAS2*↑↑, *IRF6*↑↑
Response to IFN-γ	0.001	*OAS2*↑↑, *IRF6*↑↑
Response to stimulus	0.008	*IFNA17*↑↑, *OAS2*↑↑, *IRF6*↑↑, *GNA14*↑↑, *PARP2*↓↓
B. burgdorferi sensu stricto and *B. garinii*		
Cell communication	0.002	*IL13RA1*↑↑, *MAP3K12*↑↑, *APP*↑↑, *ANGPTL2*↑↑, *IL6*↑↓
Developmental process	0.009	*IL13RA1*↑↑, *MAP3K12*↑↑, *ANGPTL2*↑↑, *IL6*↑↓
Immune system process	0.001	*OTUB1*↓↓, *IL13RA1*↑↑, *MAP3K12*↑↑, *IL6*↑↓

IFN interferon; statistical significance: $p < 0.05$; ↑, ↓: higher and lower expression in the *Borrelia*-infected NHDF cells vs. control

Borrelia spirochetes. Similar to our study, Ramesh et al. (2013) and Parthasarathy et al. (2013) found that *B. burgdorferi* induced an inflammatory response and apoptosis but in the cells of the dorsal root ganglia and oligodendrocytes. Similarly, other authors also reported that spirochetes were able to induce apoptosis in human lymphocytes that had been co-cultured with these bacteria in vitro (Perticarari et al. 2003). Interestingly, Chmielewski and Tylewska-Wierzbanowska (2011) demonstrated that caspase 3 activity increased in uninfected fibroblasts and in

cells infected with *B. afzelii* during four weeks of incubation. These authors suggested that *B. afzelii* bacteria have the ability to inhibit apoptosis for only a short period of time.

The results of Wu et al. (2015) indicated that changes in the gene expression between *Borrelia* genospecies indicate differences in their pathogenicity. The authors showed that 731 genes were differentially expressed between *B. burgdorferi* and *B. garinii* isolates that had been cultured in vitro. Our results revealed various expression profiles of the *TLR*-related genes in human cells that had been infected with *B. afzelii, B. garinii* and *B. burgdorferi* sensu stricto, which also confirm the differences in the pathogenicity of spirochetes.

In conclusion, our findings indicate that *Borrelia* has a major effect on fibroblast gene expression. Further characterization of changes in gene expression may lead to valuable insights into the role of the TLRs in the pathogenesis of Lyme disease and may provide guidelines for the development of diagnostic markers for an infection with particular *Borrelia* genospecies. Moreover, this will help to identify better treatment strategies for Lyme disease. However, further studies such as experiments with cells that are obtained from different donors and with multiple isolates of *Borrelia* spirochetes, Western Blot experiments for TLRs and in vivo research are required to confirm our findings.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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