Functional and Genomic Architecture of *Borrelia burgdorferi*-Induced Cytokine Responses in Humans

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SUMMARY

Despite the importance of immune variation for the symptoms and outcome of Lyme disease, the factors influencing cytokine production during infection with the causal pathogen *Borrelia burgdorferi* remain poorly understood. *Borrelia* infection-induced monocyte- and T cell-derived cytokines were profiled in peripheral blood from two healthy human cohorts of Western Europeans from the Human Functional Genomics Project. Both non-genetic and genetic host factors were found to influence *Borrelia*-induced cytokine responses. Age strongly impaired IL-22 responses, and genetic studies identified several independent QTLs that impact *Borrelia*-induced cytokine production. Genetic, transcriptomic, and functional validation studies revealed an important role for HIF-1α-mediated glycolysis in the cytokine response to *Borrelia*. HIF-1α pathway activation and increase in glycolysis-derived lactate was confirmed in Lyme disease patients. In conclusion, functional genomics approaches reveal the architecture of cytokine production induced by *Borrelia* infection of human primary leukocytes and suggest a connection between cellular glucose metabolism and *Borrelia*-induced cytokine production.

INTRODUCTION

Lyme disease is the most common human vector-borne disease in the United States and Western Europe, and it is transmitted through infected ticks (Oosting et al., 2016). The disease is caused by bacteria of the species *Borrelia burgdorferi sensu lato*, including *B. burgdorferi sensu stricto*, *B. afzelii*, and *B. garinii* as the major causative species. The clinical picture of Lyme disease is highly variable, ranging from early localized disease resulting in inflammation of the skin around the tick bite (erythema migrans [EM]) to disseminated infections, such as neuroborreliosis and carditis. Furthermore, chronic or persistent forms of the disease are seen, resulting in long-term inflammation of large joints or the skin. In *Borrelia*-infected tissue or cultures of human cells exposed to the pathogen, high levels of cytokines and chemokines, including IL-1β and IL-17, have been detected (Bachmann et al., 2010; Burchill et al., 2003; Kuo et al., 2011; Shin et al., 2007). A crucial step in the induction of cytokine production by monocytes or macrophages is the recognition of pathogens, which is mainly mediated by pattern recognition receptors (PRRs). In recent years, several PRRs have been described to recognize ligands of *Borrelia* microorganisms, resulting in the induction of specific cytokine responses. Toll-like receptor (TLR) 2 together with nucleotide-binding oligomerization domain (NOD) 2 were shown to play an important role in the induction of cytokines after *Borrelia* recognition, whereas TLR10 was shown to have an inhibitory role in the *Borrelia*-mediated immune responses (Oosting et al., 2010, 2011a, 2014).

A large diversity is also seen in the induction of host defense, such as antibody and cytokine responses, in *Borrelia*-infected patients. Earlier research has shown that genetic variation in patients with Lyme disease strongly affects cytokine responses, ultimately leading to variation in clinical outcome (Oosting et al., 2011b). Additionally, other studies reported that non-genetic host factors, such as age, gender, vitamin intake, nutritional status, or smoking habits, can influence susceptibility to infectious or inflammatory diseases (Fish, 2008; Franceschi and Campisi, 2014; Khoo et al., 2012; Libert et al., 2010).
Despite the importance of immune variation for the symptoms and outcome of Lyme disease, no comprehensive understanding of the genetic and non-genetic factors influencing the cytokine production induced by *Borrelia* is currently available. A missing link between unraveling the genetic factors predisposing to Lyme disease and the translation into therapeutic targets is the understanding of the functional consequences of disease-associated genetic variation. Several questions therefore arise: what is the overall pattern of cytokine responses after stimulation of human primary leukocytes with *Borrelia*, how is this impacted by genetic variation and non-genetic factors, and lastly, do these factors influence susceptibility and clinical outcome in patients?

To address these questions, we profiled *Borrelia*-induced monocyte-derived and T cell-derived cytokines in two large cohorts of 500 and 200 healthy individuals of Western European ancestry from the Human Functional Genomics Project (500 Functional Genomics Project: 500FG and 200FG cohorts). We delineated inter-individual variability of *Borrelia*-induced cytokine production in the context of other microbial challenges, as also described in studies in *Cell* by ter Horst et al. (2016) and Li et al. (2016b), and we identified genetic loci that regulate *Borrelia*-mediated immune responses. Regulatory pathways identified through this genomic approach were validated in immunologic and transcriptomic studies.

### RESULTS

**Borrelia-Induced Cytokine Production Clusters Together with Intracellular Bacteria**

To determine whether *Borrelia* spirochetes induce a pathogen-specific cytokine profile, we exposed peripheral blood mononuclear cells (PBMCs) from 500 healthy volunteers to *Borrelia burgdorferi* s.s., *Borrelia afzelii*, and *B. garinii*, *Staphylococcus aureus*, *Mycobacterium tuberculosis* (Mtbc), or *Escherichia coli* (*E. coli*) for 24 hr and 7 days. Overall, while inducing strong IL-1β and IL-6 responses, *Borrelia* induced almost no TNF-α production (Figures 1A and 1E). This was not due to experimental problems, as substantial TNF-α production was seen after stimulation with LPS (Figure 1H). Next, we assessed the correlation between *Borrelia*-induced cytokine production and cytokines induced by other intra- (i.e., Mtbc) or extracellular bacteria (i.e., *E. coli* or *S. aureus*). A strong resemblance was found between the monocyte-derived cytokine profile induced by *Borrelia* and those induced by *E. coli* and Mtbc but very little with *S. aureus*-induced cytokines (Figure S1). A different pattern emerged for the T cell-derived cytokines; *B. burgdorferi* and *Borrelia* mix cluster together, producing significant IL-22 and IL-17 but far less IFN-γ compared to the other stimuli tested (Figure 1C). Nevertheless, the strongest correlation on T cell-derived cytokines after *Borrelia* stimulation was again seen with the intracellular bacterium Mtbc (Figure 1D).

To detect the presence of high and low responders to *Borrelia* exposure, we analyzed the distribution of cytokine production. Only *Borrelia*-induced IL-1β production was found to follow a normal (Gaussian) distribution (Figure 1E), while the production of all other measured cytokines was skewed to the right (Figures 1E–1H). This may be partially explained by the substantial amount of low responders (cytokine production below detection limit) seen for TNF-α, IL-17, and IFN-γ.

### The Role of Non-genetic Factors for *B. burgdorferi*-Induced Cytokine Production

It has already been known that lifestyle influences immune responses, specifically cytokine production upon exposure to pathogens (Franceschi and Campisi, 2014; Libert et al., 2010). To detect differences in cytokine production after *Borrelia* exposure, we analyzed several factors, including previous exposure to tick bites, gender, body mass index (BMI), smoking habits, age, and vitamin D serum levels using a linear mixed model. Previous exposure to tick bites did not influence *Borrelia*-induced cytokine production (Figure 2A) regardless of the number of bites (data not shown). Stratifying on gender, BMI, smoking habits, and levels of circulating vitamin D did not affect cytokine production either (Figure 2A). Of high interest, however, age was found to have an important impact on *Borrelia*-induced cytokines in T cells (Figure 2A). PBMCs of elderly individuals produced significantly less IL-22 and IFN-γ after 7 days of *Borrelia* exposure, while no age-dependent effect was observed on IL-17 concentrations (Figure 2B). The same pattern was seen for Mtbc stimulation, further strengthening the resemblance between the immune responses to *Borrelia* and Mtbc. To determine whether previous exposure to *Borrelia* alters cytokine production upon (re-)stimulation with *B. burgdorferi*, *M. tuberculosis*, *S. aureus*, or *E. coli*, we stratified healthy individuals from the 200FG cohort based on the presence or absence of antibodies (IgG or IgM) against *Borrelia* (Figures 2C–2F; Figure S2). No differences in cytokine production, either monocyte-derived or T cell-derived, could be detected between individuals having positive or negative serology to *Borrelia*.

**Genome-wide Mapping Identifies *Borrelia*-Specific eQTLs**

Genetic factors have been described to influence cytokine production by immune cells and are able to strongly influence clinical outcome in disease (Netea et al., 2012). Therefore, we assessed genetic factors that specifically influence *Borrelia*-induced cytokine production. Cytokine production quantitative trait loci (cQTLs) analysis was performed as described (Li et al., 2016a). To correct for multiple testing, we set the threshold for genome-wide statistical significance at p < 5 × 10⁻⁸. However, using this threshold, only two cQTLs achieved genome-wide significance: rs17615278, located in the non-coding RNA transcript RP11-92J19.3, and rs11103976, located in MGAT4C, which codes for a glycosyltransferase protein. Both of these expression QTLs (eQTLs) were found for *Borrelia burgdorferi*-induced, but not *Borrelia*-mix-induced, IFN-γ production (Figure 3A). To increase our sensitivity for eQTLs affecting *Borrelia*-mix-induced cytokine production, we also analyzed cQTLs with p values between 1 × 10⁻⁵ and 1 × 10⁻⁸ for IL-22, IFN-γ (Figures 3B and 3C), IL-1β, and IL-6 (Table S1). To rule out that these eQTLs were found by chance, we performed validation in a separate cohort of healthy individuals (200FG), 55 top cQTLs for *Borrelia*-induced cytokines (Table S1) were also analyzed in the 200FG cohort, and 54/55 could be replicated for at least one cytokine (nominal p value < 0.05), including the two genome-wide significant cQTLs.

**Expression QTL Mapping Reveals Genes Involved in *Borrelia*-Induced Cytokine Production**

To identify the causal genes influenced by the detected cQTL SNPs, we performed eQTL mapping using RNA sequencing...
Figure 1. Similarities and Differences of *Borrelia* to Several Other Bacteria

(A) Principal Component Analysis (PCA) bi-plot based on the monocyte-derived cytokines (IL-6, TNF-α, and IL-1β). Each dot represents one individual’s cytokine response against a particular stimulus; the colors indicate the different stimuli. The vectors indicate the importance of the original variables (cytokines) in creating the PCA axis system. The closer the points cluster along a vector, the higher the production for that cytokine.

(B) Correlation coefficients after 24 hr of stimulation with *S. aureus*, *M. tuberculosis*, *E. coli*, *Borrelia mix*, and *B. burgdorferi* for the monocyte-derived cytokines IL-1β, IL-6, and TNF-α, calculated using Spearman rank correlation.

(C) PCA bi-plot based on the T cell-derived cytokines (IL-17, IL-22, and IFN-γ).

(D) Correlation coefficients after 7 days of stimulation with *S. aureus*, *M. tuberculosis*, *Borrelia mix*, and *B. burgdorferi* for the T cell-derived cytokines IL-17, IL-22, and IFN-γ.

(E and F) Distributions of the monocyte-derived (E) or T cell-derived (F) cytokine responses for *B. burgdorferi*. The red lines indicate upper and lower thresholds of the ELISA measurement, and the black line indicates the best fitting theoretical Gaussian distribution.

(G) Heatmap showing the distribution of cytokine levels from the 500FG cohort. Red indicates normal distribution and white indicates non-normal distribution according to the Shapiro-Wilk normality test. Gray indicates that these cytokine distributions could not be tested.

(H) TNF-α production (pg/mL) after 24 hr stimulation with lipopolysaccharide (LPS).
data from 629 subjects in another separate cohort, the LL-DEEP cohort (Tiqchelaar et al., 2015). We analyzed the top 20 cQTLs per Borrelia-induced cytokine and found 46 eQTLs (Table S1). Pathway analysis indicates that the eQTL genes are mainly involved in regulating general cellular responses, such as development and response to chemicals but also detection of bacterial lipopeptides (Table S2). However, enrichment analysis showed no specific pathway to be significantly overrepresented after correction for multiple testing. We therefore continued by assessing the eQTLs individually. The most interesting eQTL identified was SNP rs55710213, which affects the mRNA expression of HIF1AN, encoding for the factor inhibiting HIF-1α (FIH-1), as seen in Figure 3D. This SNP significantly impacts IL-22 levels in PBMCs stimulated with Borrelia species while having less effect on Mtb and no effect at all on S. aureus stimulations (Figure 3F). Effect of this SNP was also seen, although weaker, on IL-17 production, but not in IFN-γ production (Figures 3F and 3G).

**HIF-1α Regulation of Glucose Metabolism Is a Crucial Process for Borrelia-Induced Cytokine Production**

We identified a SNP regulating the expression of the factor inhibiting HIF-1α as an important variable in Borrelia-induced cytokine production. HIF-1α is a transcription factor with a crucial role in regulating glucose metabolism, the response to hypoxia, and to antigenic stimulation (Sakamoto and Seiki, 2010; Scholz et al., 2016; Zhang et al., 2010). This suggests that cellular glucose metabolism may be important in Borrelia-induced cytokine production. To investigate this, we performed transcriptomic analysis on Borrelia-stimulated PBMCs from a previously published study (Smeekens et al., 2013). These data revealed significant upregulation of enzymes associated with the citric acid (TCA) cycle and transcription factors involved in the metabolism after stimulation. Corresponding to the transcriptomic data, PBMCs stimulated with Borrelia were shown to consume...
These findings indicate that Borrelia indeed modulates glucose metabolism in PBMCs, mostly by increasing the glycolysis pathway.

Previously, the PI3K/Akt/mTOR pathway was shown to be involved in initiating the metabolic switch toward aerobic glycolysis (Warburg effect) after stimulation (Gerriets and Rathmell, 2012; Weichhart et al., 2015). To test whether this is also the case after Borrelia stimulation, we analyzed protein expression of p-AKT and the downstream target of mTOR, p-4EBP1. As shown in Figure 4F, Borrelia stimulation increased activation of these signaling molecules, thereby supporting a role for the involvement of the PI3K/mTOR signaling pathway. To assess the role of the glucose metabolism pathways for Borrelia-induced cytokine production, we stimulated PBMCs with Borrelia in the presence of pharmacological inhibitors. Pretreatment of the cells with the glycolysis inhibitor 2-deoxyglucose (2-DG) drastically decreased production of IL-22 induced by Borrelia (Figure 4G). This effect was also seen for IL-1β, TNF-α, and IL-6 and the T cell-derived cytokines IFN-γ and IL-17 (Figures S3A–S3E). Conversely, complete shutdown of oxidative phosphorylation (OXPHOS) by the combined action of rotenone and antimycin A had no effect on cytokine production (Figure 4G, second panel). All inhibitors were checked for cytotoxicity by Annexin V/PI flow cytometry staining to rule out cell death as a confounding factor (Figure S4A).

Additionally, to analyze whether the PI3K/mTOR pathway is also involved in Borrelia-induced cytokine production, we treated the cells with the Akt-inhibitor wortmannin and the mTOR-inhibitors rapamycin and Torin1 and analyzed the production of IL-22 (Figure 4G). Interestingly, while mTOR inhibition significantly decreased IL-22 production, Akt inhibition actually showed a trend toward increasing IL-22 production. This may indicate uncoupling of these pathways in the case of Borrelia stimulation. Inhibiting HIF-1α significantly decreased production of IL-22 as well; however, this was accompanied by a substantial amount of cell death, as determined by Annexin V staining (Figure S4B). To further strengthen the association between HIF-1α-induced glycolysis and the immune response against Borrelia, we performed experiments on murine cells with an HIF-1α deficiency in the myeloid lineage. As seen in Figures 5A and 5B, cytokine production was reduced in HIF-1α knockout bone-marrow-derived macrophages (BMDMs) compared to...
Figure 4. Borrelia-Induced Changes in Glucose Metabolism
(A) Heatmap of microarray data depicting genes in the glycolysis and TCA cycle pathway significantly affected by Borrelia stimulation in healthy PBMCs (n = 48).
(B) qPCR confirmation of a representative selection of genes in healthy PBMCs (n = 7) stimulated with Borrelia.
(C) Graphical representation of enzymes and transcription factors in central metabolic pathways, whose expression was significantly affected by Borrelia stimulation (green arrows, upregulation; red arrows, downregulation). Figure was designed in the MindtheGraph platform. *p < 0.05, paired t test.
(D) Functional analysis of glycolysis pathway activity measured by glucose consumption and lactate production after several time points and confirmed by measuring extracellular acidification rate (ECAR) in healthy PBMCs after 24 hr stimulation with B. burgdorferi (B.b.) (n = 6).

(legend continued on next page)
Production of innate cytokines, TNF-α (A) and IL-6 (B), and lactate (C) was determined from cell culture supernatants of HIF-1α−/− murine bone-marrow-derived macrophages after 24 hr of stimulation with Borrelia burgdorferi (10^6 spirochetes/mL). Production of T cell-derived cytokines, IL-22 (D) and IL-17 (E), and lactate (F) was determined in cell culture supernatants from murine spleen cells with an HIF-1α knockout in the myeloid lineage (n = 5) after 6 days of stimulation with Borrelia burgdorferi (10^6 and 10^7 spirochetes/mL, respectively), Candida albicans (C.a.), or anti-CD3/anti-CD28 antibodies.

**DISCUSSION**

Cytokines are a crucial component of the immune system, and they play an important role in both the antimicrobial host defense, as well as in the pathophysiology of immune-mediated diseases. This is also the case for Lyme disease, but little is known regarding the variability in these responses between individuals. The Human Functional Genomics Project investigates the variability of human immune responses in general, with a specific interest in cytokine responses. We report the overall impact of environmental (ter Horst et al., 2016) and genetic (Li et al., 2016b) host factors, as well as the microbiome (Schirmer et al., 2016) for modulating cytokine responses to microbial and non-microbial stimulation. In the present study, we focused...
on deciphering the functional and genetic architecture of the Borrelia-induced cytokine responses.

The general cytokine profile in PBMCs stimulated with two preparations of Borrelia and several other pathogens was assessed. It should be noted that this model reflects the systemic response to the bacterium rather than the initial inflammatory response, which occurs locally in the skin. No large differences were seen between the responses to Borrelia alone or the Borrelia mix. When comparing with other bacterial stimuli, the Borrelia-induced cytokine profile most closely resembled that of Mtb. Biologically, this is an important observation, as Mtb and Borrelia are both recognized by a combination of both membrane-bound (TLR2) and intracellular (NOD2) receptors, while the extracellular bacteria are largely recognized by membrane-bound receptors only (Kleinnijenhuis et al., 2011, 2012; ter Horst et al., 2008; Nelson et al., 2015), though it is difficult to distinguish biological age-related factors from behavioral factors. Additionally, several disseminated Lyme disease symptoms, such as acrodermatitis chronica atrophicans (ACA), are known to be more prevalent in elderly individuals (Mullegger, 2004).

Next, we assessed the effect of non-genetic host factors on cytokine responses to Borrelia. These appeared to play a limited role, with the notable exception of age. Increasing age strongly decreased Borrelia-dependent IL-22 production, and this effect was more pronounced for Borrelia than for other stimuli (ter Horst et al., 2016). This may have important implications, as this could indicate higher susceptibility to Lyme disease in the elderly. It is already known that the incidence of Lyme disease shows a bimodal age distribution with a peak incidence in individuals aged 50–70 (Berglund et al., 1995; Fuchs and Poggensee, 2008; Nelson et al., 2015), though it is difficult to distinguish biological age-related factors from behavioral factors. Additionally, several disseminated Lyme disease symptoms, such as acrodermatitis chronica atrophicans (ACA), are known to be more prevalent in elderly individuals (Mullegger, 2004).
Surprisingly, neither previous exposure to tick bites, nor previous encounters with the spirochete, defined by the presence of *Borrelia* antibodies, influenced the cytokine response. Lastly, one of the most important aims of the present study was the identification of the genomic architecture of cytokine responses in immune cells upon stimulation with *Borrelia*. Therefore, a genome-wide association of eight million SNPs with *Borrelia*-specific induction of cytokines was performed, and cQTLs influencing both monocyte-derived and T cell-derived cytokines were identified. Most of these cQTLs encode for genes with unknown function for the cytokine responses to *Borrelia*, and follow-up studies are warranted to investigate their role. It should be noted that different eQTLs were found for *Borrelia burgdorferi*- and *Borrelia* mix-induced cytokines, indicating that different pathways may be involved in the immune response against the different *Borrelia* species. This is also reflected by the fact that the different species are known to induce different symptoms (van Dam et al., 1993).

A very interesting link was identified between cellular glucose metabolism and *Borrelia*-induced cytokine production. One of the most significant eQTLs was found for a SNP regulating the transcription of *HIF1AN*, encoding for the factor inhibiting HIF-1α (FIH-1), which significantly affected *Borrelia*-induced IL-22 levels. HIF-1α is a transcription factor with a crucial role in regulating glucose metabolism (Sakamoto and Seiki, 2010; Scholz et al., 2016; Zhang et al., 2010). In line with this, *Borrelia* was found to induce a shift in cellular metabolism toward a Warburg effect: a switch from oxidative phosphorylation to aerobic glycolysis, mediated by the mTOR/HIF-1α pathway. This switch has been previously reported to be involved in cytokine production during activation of Th1 (Cham and Gajewski, 2005; Chang et al., 2013) and, more recently, also Th17 cells (Gerriets et al., 2015; Michalek et al., 2011) and NK-cells (Keating et al., 2016). Interestingly, although many previous studies showed the involvement of the PI3K/Akt pathway in activating mTOR, our data indicate that IL-22 production after *Borrelia* stimulation was Akt independent, yet highly dependent on mTOR. This indicates that mTOR is activated through an unknown alternative pathway. Although uncommon, similar findings have been previously reported (Brewer et al., 2007). Nevertheless, our in vitro data showed that the induction of glycolysis through the mTOR/HIF-1α pathway is essential for *Borrelia*-induced production of IL-22, as well as other cytokines. Furthermore, experiments in HIF-1α−/− bone-marrow-derived macrophages showed that both cytokine production and lactate production were decreased compared to WT cells. This strongly supports a role for HIF-1α-mediated glycolysis in *Borrelia*-induced cytokine production. Altogether, our data provides evidence that the glycolysis pathway is physiologically relevant in the human immune response. Our findings showing a stronger effect of the *HIF1AN* eQTL on IL-17 and IL-22 rather than on IFN-γ are supported by Michalek et al. (2011), showing a higher glycolytic rate in Th17 cells compared to Th1 cells, which could explain the higher sensitivity to changes in glycolytic flux. In addition, Gerriets et al. (2015) provided evidence for an important role for PDH1, present in Th17 cells but not in Th1 cells, for glycolysis and inflammation. Also, transcriptional profiling of leukocytes of patients with Lyme disease resulted in significantly upregulated genes in the mTOR-HIF-1α and glycolysis pathway in white blood cells shortly after *Borrelia* stimulation. In line with these findings, we found that serum lactate was elevated 2 weeks after initial diagnosis of *Borrelia* infection. Furthermore, lactate levels in cerebral spinal fluid (CSF) were previously shown to be strongly increased in 63% of Lyme patients with facial palsy, while CSF lactate was normal in patients with facial palsy due to other infections (Kindler et al., 2015). Similarly, CSF lactate was elevated in patients with acute Lyme neuroborreliosis, but not in patients with neurosyphilis (Djukic et al., 2012). These data show that a shift of immune cell metabolism toward glycolysis occurs both in vitro and in vivo after an encounter with *Borrelia*. This indicates the involvement of a cellular glucose metabolism pathway in the pathogenesis of Lyme disease and possibly a target for drug development in long-term immunemediated complications.

One of the strongest eQTLs was found for a SNP in MGAT4C involved in protein glycosylation (Mak et al., 2011), affecting *Borrelia*-induced IFN-γ responses. Glycosylation is known to be crucial for protein function, and recent studies identified defects in protein glycosylation causing profound immunodeficiency (Lyons et al., 2015). In addition, very recently it was reported that MGAT enzymes were linked to metabolic reprogramming (Ryczko et al., 2016), further supporting the role for metabolism in *Borrelia*-induced cytokine production. It is thus tempting to speculate that protein glycosylation might also be crucial for anti-*Borrelia* host defense mechanisms.

In conclusion, we used a functional genomics approach to describe the architecture of cytokine responses induced by *B. burgdorferi* in two large cohorts of healthy volunteers. We identified several crucial characteristics of *Borrelia* cytokine responses, such as a strong correlation with cytokine responses induced by intracellular bacteria, a strong influence of age on *Borrelia*-induced IL-22, and several genomic factors influencing these responses. Additionally, we demonstrate that a switch in glucose metabolism in immune cells is crucial for induction of cytokines by *Borrelia*. Future studies should aim to unravel the function of other cQTLs and genes identified here for the immune responses during Lyme disease.

**EXPERIMENTAL PROCEDURES**

**Borrelia burgdorferi, Borrelia afzelii, and Borrelia garinii Cultures**

*Borrelia* species were cultured at 25°C (and in a later time point at 34°C) as described in the Supplemental Experimental Procedures.

**Escherichia coli**

*E. coli* ATCC 25922 was grown overnight in culture medium, washed three times with PBS, and heat-killed for 60 min at 80°C. Aliquots were stored at −80°C throughout the study.

**Staphylococcus aureus**

*S. aureus* strain ATCC 29213 was grown overnight in culture medium, washed twice with cold PBS, and heat-killed for 30 min at 100°C. Aliquots were stored at −80°C throughout the study.

**Mycobacterium tuberculosis**

Cultures of H37Rv/C14 M. *tuberculosis* were grown to mid-log phase in Middlebrook 7H9 liquid medium (see Supplemental Experimental Procedures).

**Borrelia Serology**

*Borrelia* antibodies were measured using specific ELISAs (Serion/Virion Borrelia IgG; ESR-121-G and Borrelia IgM; ESR-121-M) and western blot analysis
(Eurolmmun Borrelia IgG and IgM, DY-2131-3001-1G and DY-2131-3001-1M), both according to the manufacturer’s instructions.

Ethics Statement
The 500 functional genomics study (500FG) was approved by the Ethics Committee of Radboud University Nijmegen (nr. 42561.091.12). Experiments were conducted according to the principles expressed in the Declaration of Helsinki.

500FG and 200FG Cohorts
500FG is a cohort of 500 healthy individuals of Dutch European ancestry from the Human Functional Genomics Project (www.humanfunctionalgeneomics.org). The cQTLs identified were validated in a second cohort of 200 healthy volunteers working as foresters in the Netherlands. Volunteers were asked to donate blood in order to determine the serum antibody response against Borrelia, since Lyme disease occurs as an occupational disease. None of the volunteers had an active Borrelia infection. In this cohort, all individuals gave written informed consent to donate an additional blood sample for research use. General characteristics of the cohorts have been described by ter Horst et al. (2016).

Isolation of Human PBMCs and Stimulation of Cytokine Production
Venous blood was drawn from the cubital vein of volunteers into 10 mL EDTA tubes (Monoject). Isolation of PBMCs was performed using Ficoll isolation, as described in the Supplemental Experimental Procedures. For measurements of metabolic parameters, see Supplemental Experimental Procedures.

Cytokine Measurements
Human IL-1β, TNF-α, IL-22, IL-17, IFN-γ, and IL-6 were determined by using commercial ELISA kits (PeliKine Compact, Sanquin, or R&D Systems) according to the manufacturer’s instructions. The sensitivity of all assays was 20 pg/mL.

Genotyping, Quality Control, and Imputation
DNA samples of 500 individuals were genotyped using the commercially available SNP chip, Illumina HumanOmniExpressExome-8 v1.0. The genotype calling was performed using Optical 0.7.0 (Shah et al., 2012) using the default settings. Samples with a call rate ≤ 0.99 (n = 18) were excluded from the dataset as were variants with an HWE ≤ 0.0001 and MAF ≤ 0.001. Potential ethnic outliers, identified by multi-dimensional scaling plots of our samples merged with 1000 Genome data (Figure S9), were also excluded (n = 17). This resulted in a dataset of 442 samples containing both cytokine and genotype information of 518,980 variants for further imputation. The strands and variant identifiers were aligned to the reference Genome of the Netherlands (Genome of the Netherlands Consortium, 2014) dataset using Genotype Harmonizer (Deelen et al., 2014). The data were phased using SHAPEIT2 v2.r644 (Delanear et al., 2013) and imputed using IMPUTE2 (Howie et al., 2011) using the GoNL as the reference panel (Genome of the Netherlands Consortium, 2014). Post-imputation provided 7,512,899 variants. We selected 4,242,453 SNPs that showed MAF ≥ 5%, INFO score > 0.8, and 3 samples per genotype for downstream cytokine QTL mapping.

Cytokine QTL Mapping
We used the 500FG dataset as a discovery cohort to identify genome-wide significant cQTLs since this cohort had the largest numbers of individuals (n = 489) in which both Borrelia-stimulated cytokine data and genome information was available. The 200FG dataset (n = 78) was used as validation cohort. See Supplemental Experimental Procedures.

RNA Sequencing, eQTL Expression, and Pathway Analysis
Candidate genes from significant cytokine QTL loci were further tested for responsiveness to any of the pathogens using RNA sequencing. See Supplemental Experimental Procedures for detailed information.

Microarray and qPCR
We obtained previously published transcriptome data for our analysis (GEO: GSE42606) (Smeekens et al., 2013). See Supplemental Experimental Procedures.

Cell Viability and Metabolite Measurements
Cell survival was analyzed in Borrelia- and medium-stimulated PBMCs using Annexin V/PI staining as described in the Supplemental Experimental Procedures. Lactate and glucose concentrations in cell culture supernatants were quantified using Amplex Red reagent (Thermo Fisher Scientific).

Extracellular Flux Measurements
Extracellular acidification rates (ECAR) and oxygen consumption rates (OCR) were measured using Seahorse XF™96 analyzer (Seahorse Bioscience).

Western Blotting
Western blotting was performed using a TransTurbioBlot system (Bio-Rad; Supplemental Experimental Procedures).

HIF-1α Knockout Experiments
Bone marrow and spleen cells from mice with an HIF-1α deficiency in the myeloid cell lineage were obtained from Dr. R.A. Cramer (Dartmouth, NH, USA). Spleen cells were stimulated with B. burgdorferi (107 and 108 spirochetes/mL, respectively), Candida albicans (C.a.), or anti-CD3/anti-CD28 antibodies for 6 days, after which supernatants were collected (Supplemental Experimental Procedures).

Transcriptome Analysis and Serum Lactate Measurements in Lyme Patients
RNA sequencing data of acute Lyme patients were obtained from a publicly available dataset (Bouquet et al., 2016) (GEO: GSE63085). Expression of selected genes was compared among EM patients at different time points and healthy controls by Kruskal-Wallis one-way ANOVA with Dunn’s post hoc t test. Serum lactate concentrations were determined in a Romanian cohort of clinically proven EM patients and tick-bitten healthy controls. All patients were included before start of antibiotic treatment (conform IDSA guidelines), and samples were collected at baseline, after 2 weeks, and after 6 weeks.

Statistical Analysis
The data are expressed as mean ± SEM unless mentioned otherwise. Differences between experimental groups were tested using the Mann-Whitney U test performed on GraphPad Prism 4.0 software (GraphPad). p values of ≤ 0.05 were considered significant.

SUPPLEMENTAL INFORMATION
Supplemental Information includes Supplemental Experimental Procedures, five figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.chom.2016.10.006.

AUTHOR CONTRIBUTIONS

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