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The Joint-Specific Expression Profile of Borrelia Burgdorfri Spirochetes in the Murine Hosts

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ABSTRACT

Objective: To identify the joint-specific expression profile of *Borrelia burgdorfri* in the murine hosts.

Methods: Establishing the murine model of Lyme disease, sacrificing the infected mice at different time-points, collecting the joint, heart, skin, and urinary bladder samples of the infected mice, and extracting total RNA from the samples. DECAL technique and microarray were applied to brain the transcriptomes of *Borrelia burgdorferi* in the joints, heart, skin, and urinary bladder, the transcriptomes from different tissues were compared to identify the joint-specific expression profile of *Borrelia burgdorferi*.

Results: *Borrelia burgdorferi* expresses 21 joint-specific genes, including 13 genes located in chromosome and 9 genes located in plasmids at day 15 after infection, and expresses 24 genes, including 13 genes in chromosome and 11 genes in plasmids at day 105 after infection. *Borrelia burgdorferi* shows specific gene expression profile in the murine joints.

Conclusion: *Borrelia burgdorferi* expresses some specific genes different from those in the other tissues in infected murine joints. These joint-specific genes might were involved in the survival and arthritis pathogenesis of *Borrelia burgdorferi*.

Keywords: *Borrelia burgdorferi*; Lyme disease; DECAL; Gene microarray; Transcriptome; Expression profile

Lyme borreliosis, caused by *Borrelia burgdorferi* [1], can involve the skin, joints, heart, and nervous system [2]. When *Ixodes scapularis* ticks feed on mice or humans, spirochetes are deposited into the skin. *B. burgdorferi* replicate in the dermis and then disseminate to distant cutaneous sites and other organs, including the joints [3]. Lyme arthritis occurs in a substantial number of untreated patients [4], often several weeks to months after the tick bite, and is associated with spirochete invasion of the joints [5]. In most cases antibiotic therapy is curative; however, some patients develop a form of antibiotic-resistant arthritis that is thought to be unrelated to persistent infection [6].

The understanding of Lyme disease has been aided by several animal models, most notably the murine model. C3H/He mice infected with *B. burgdorferi* develop severe joint swelling which partially mimics human disease and has been helpful for understanding the pathogenesis of Lyme arthritis. The presence of *B. burgdorferi* in murine joints, and the innate and adaptive host responses to the pathogen all contribute to the development of inflammation [3]. Spirochete genes are also implicated in murine Lyme arthritis as *B. burgdorferi* lacking certain plasmids are less arthritogenic. Indeed *B. burgdorferi* Arp is expressed in many tissues during infection and antisera against Arp can attenuate murine Lyme arthritis [7,8]. Specific spirochete antigens that are preferentially induced in the joints and causally associated with the genesis of arthritis have, however, not yet been identified.

The preferential up regulation of specific *B. burgdorferi* genes throughout the spirochete life cycle - both in the vector and mammalian host - plays an important role in pathogen survival [9-11]. It is likely that the diverse metabolic or immune microenvironments within a variety of mammalian tissues may influence the ability of *B. burgdorferi* to persist within various host tissues [12-14]. We hypothesized that, therefore, particular *B. burgdorferi* genes are selectively expressed in murine joints, and these gene products contribute to spirochete colonization of the joints and the development of arthritis. Characterization of microbial ligands that are expressed in a tissue-specific manner is critical for understanding the pathogenesis of complex infectious diseases.

The relatively few number of *B. burgdorfei* spirochetes in the mammalian hosts has r limited our ability to directly examine spirochete gene expression *in vivo*. To overcome this limitation, we have exploited differential expression analysis by using a custom-amplified library (**DECAL**) [15,16]. DECAL, a technique to selectively amplify specific prokaryotic transcriptomes, was first used for the global analysis of gene expression in *Mycobacterium tuberculosis* grown *in vitro* [16]. The technique can be performed with as little as 10 ng of total bacterial RNA, can detect as low as 4-fold differences in gene expression, and can be used where contaminating host material is present [16]. Recently, *B. burgdorferi* whole-genome microarrays have been used to examine the influence of pH and temperature on the transcriptome of spirochetes grown *in vitro* [17,18]

and of spirochetes grown *in vivo* within dialysis membrane chambers (**DMCs**) [19]. Therefore, DECAL was exploited in conjunction with whole-genome membrane arrays (Brooks *et al*) [18] to compare the transcriptome of *B. burgdorferi* in the joint, heart, skin, and urinary bladder in the murine model of Lyme borreliosis.

MATERIALS AND METHODS

Microarray and Bb-CAL Construction

A whole-genome microarray containing 1,697 putative ORFs encoded by the *B. burgdorferi* B31 genome was printed onto a positively charged nylon membrane, as described [18]. A custom-amplified *B. burgdorferi* library was constructed as described [15]. Briefly, a ZAP II *B. burgdorferi* genomic library was screened with radioactively labeled *B. burgdorferi* rRNA probes and hybridizing clones discarded. The nonribosomal clones were pooled and restriction digested with EcoRV and SmaI. The 200- to 2,000-bp fragments were gel-purified, ligated to PCR adapters, and PCR-amplified, amplified PCR products are Bb-CAL.

Spirochete Preparation

B. burgdorferi B31 clone 5A11 (a gift from Steven Norris, University of Texas, Houston, TX) was used in the all experiments. A frozen aliquot of *B. burgdorferi* was expanded in Barbour-Stoenner-Kelly H complete medium at 33˚C (Sigma Chemical Co., St. Louis, MO). Spirochetes were grown to mid-log phase, assessed for viability, and then counted by dark-field microscopy using a bacterial counting chamber. Inocula were diluted to obtain 105 spirochetes in 0.1 ml of BSKII medium.

Mice and Animal Experiment

Ten Specific-pathogen-free, 5-week-old C3H/HeJ (C3H) mice were obtained from The Jackson Laboratory, Bar Harbor, Maine, 8 mice were used for infection experiments, 2 mice used for negative control. Mice were given one single intradermal injection of 105 spirochetes. Four Mice that were infected with *B. burgdorferi* spirochetes and 1 control mouse were sacrificed 15 days and 105 days after infection. Urinary bladders, hearts, joints, and dorsal skins (not from the inoculation site) were harvested and immediately frozen in liquid nitrogen. Frozen samples were stored at -80˚C until total RNAs were isolated.

RNA Isolation

RNAs were isolated from the joint, heart, skin and bladder tissue of *B. burgdorferi*-infected mice (25mg / each tissue) and control mice by using the RNAWIZ RNA isolation reagent (Ambion, Austin, TX) according to the manufacturer's protocol. One microgram of total RNA was used to prepare biotinylated cDNA by using biotinylated random hexamer primers, biotin-dATP, and the Super Script First Strand synthesis system for reverse transcription (Life Technologies, Gaithersburg, MD), essentially as described [19].

Positive Selection and Amplification

Total RNA samples isolated from joints, heart, skin, and bladder of *B. burgdorferi*-infected mice and control mice were reverse transcribed in the presence of biotin-dATP, separately. The biotinylated cDNA samples were hybridized to Bb-CAL under stringent conditions. Equal amounts of biotinylated cDNA prepared from joints, heart, skin and hebladder (normalized based on the levels of flaB transcripts) were hybridized separately to Bb-CAL and the biotinylated cDNA-Bb-CAL hybrids bound to streptavidin-coated magnetic beads (Dynal, Lake Success, NY). The Bb-CAL bound to the beads was eluted by boiling and PCR-amplified by using Uniamp primers, as described [16]. The PCR products (joint-Bb-DECAL, Heart-Bb-DECAL, skin-Bb-DECAL, and bladder-Bb-DECAL) represent *B. burgdorferi* transcripts expressed in the joints, heart, skin and bladder tissues.

Differential Hybridization Analysis Using Whole-Genome Arrays

Equal amounts of PCR products corresponding to joint-Bb-DECAL, Heart-Bb-DECAL, skin-Bb-DECAL, and bladder-Bb-DECAL (template DNA normalized based on the level of flaB amplicon obtained by using specific PCR primers) were randomly labeled with [-32P]. The radio labeled probes were also normalized based on their hybridization intensity to equal amounts of flaB PCR product blotted onto nylon membrane. The normalized probes were then used to probe duplicate arrays by using Rapid Hyb buffer (Amersham Pharmacia-Pharmacia) according to the manufacturer's protocol. Hybridization was scored visually. Spots were given a score from 0 to 3 based on the intensity of hybridization, the results tabulated, specific tissue-related transcriptomes of two time-points were obtained.

Determination of Joint-Specific Expression Profile of *Borrelia Burgdorfri*

Tissue-related transcriptomes at the same time-points were compared, all the genes which only express in the joint tissue, but not in the other tissues were selected, and tabulated, and called as joint-specific expression profile of *Borrelia burgdorfri* at the specified time-point.

Bioinformatics

The predicted proteins of selected *B. burgdorferi* sequences were analyzed by using the PSORT program (www.psort.org) to assess the probability for the input protein to be localized at the inner membrane (**IM**), outer membrane (**OM**), periplasmic, and cytosolic sites. The predicted proteins of selected *B. burgdorferi* sequences were also analyzed against the PROSITE database (http://ca.expasy.org/prosite) to determine the presence of lipid attachment and processing sites to establish that the input proteins encoded potential lipoprotein.

RESULTS

The Joint-Specific Expression Profile of *Borrelia Burgdorfri* in the Murine Hosts 15 Days after Infection

By comparing the transcriptomes of *Borrelia burgdorfri* in the murine joints, heart, skin, and urinary bladder 15 day after infection, we found that *Borrelia burgdorfri* spirochete expresses 21 joint-specific genes, of which 13 genes are located in chromosome, 8 genes are located in plasmids (Table 1).

Table 1: Joint-specific expression profile of *Borrelia burgdorfri* 15 days after infection.

Gene name	Description of gene product
Genes located in chromosome	
BB0014	priA, primosomal protein
BB0016	glpE protein
BB0021	S-adenosylmethionine:tRNA ribosyltransferase isomerase
BB0210	lmpl, surface-located membrane protein
BB0235	GTP-binding protein
BB0300	ftsA, cell division protein
BB0363	periplasmic protein
BB0382	BmpB, basic membrane protein B
BB0383	BmpA, basic membrane protein A
BB0574	ntegral membrane protein
BB0660	GTP-binding protein
BB0678	rbsC-1, permease protein
BB0731	hypothetical protein
Genes located in plasmids	
BBF01	Arp, arthritis-related protein
BBH33	hypothetical protein
BBJ09	OspD, outer suface protein D
BBK33	hypothetical protein
BBO06	hypothetical protein
BBP31	hypothetical protein
BBQ26	hypothetical protein
BBQ72	hypothetical protein

The Joint-Specific Expression Profile of *Borrelia Burgdorfri* in the Murine Hosts 105 Days after Infection

By comparing the transcriptomes of *Borrelia burgdorfri* in the murine joints, heart, skin, and urinary bladder 105 day after infection, we found that *Borrelia burgdorfri* spirochete expresses 24 joint-specific genes, of which 13 genes are located in chromosome, 11 genes are located in plasmids (Table 2).

Table 2: Joint-specific expression profile of *Borrelia burgdorfri* 105 days after infection.

Gene name	Description of gene product
Genes located in chromosome	
BB0023	ruvA, Holliday junction DNA helicase
BB0228	hypothetical protein
BB0237	apolipoprotein N-acyltransferase
BB0335	oppF
BB0342	gatA, Glu-tRNA amidotransferase
BB0380	mgtE, Mg^{++} transport protein
BB0382	bmpB
BB0383	bmpA
BB0394	nusG, transcriptin antitermination factor
BB0446	aspS, aspartyl-tRNa sythetase
BB0528	hypothetical protein
BB0574	integral membrane protein
BB0660	GTP-binding protein
Genes located in plasmids	
BBA36	hypothetical protein
BBA72	hypothetical protein
BBF01	arp, arthritis-related protein
BBH27	hypthetical protein
BBK33	hypthetical protein
BBL21	hypthetical protein
BBM21	hypthetical protein
BBM36	hypthetical protein
BBP21	hypthetical protein
BBS06	hypthetical protein
BBU03	hypthetical protein

DISCUSSION

B. burgdorferi preferentially expresses specific genes throughout its life cycle, both in the arthropod vector (*Ixodes scapularis*) and in the vertebrate host. It is generally considered that this process of differential gene expression facilitates spirochetal survival and disease pathogenesis. *B. burgdorferi* gene expression is apparently orchestrated by environmental factors, including pH, temperature, and host immune responses. The analysis of tissue-specific expression of a limited number of genes has shown that *B. burgdorferi* also differentially express antigens in diverse tissues within the vertebrate host and the tick vector [15,19] invoking the additional role of physiological factors other than pH and temperature in modulating *B. burgdorferi* gene expression. The relatively few number of organisms in the mammalian hosts has limited our ability to directly examine spirochete gene expression. To overcome this limitation, we have exploited differential expression analysis by using a custom-amplified library (**DECAL**) [15,16]. DECAL, a technique to selectively amplify specific prokaryotic transcriptomes, was first used for the global analysis of gene expression in *Mycobacterium tuberculosis* grown *in vitro* [16]. The technique can be performed with as little as 10 ng of total bacterial RNA, can detect as low as 4-fold differences in gene expression, and can be used where contaminating host material is present [16]. Recently, *B. burgdorferi* whole-genome microarrays have been used to examine the influence of pH and temperature on the transcriptome of spirochetes grown *in vitro* [18] and of spirochetes grown *in vivo* within dialysis membrane chambers (**DMCs**) [20]. Therefore, DECAL was exploited in conjunction with whole-genome membrane arrays (Brooks *et al*) [20] to compare the transcriptome of *B. burgdorferi* in the joint, heart, skin, and bladder in a murine model of Lyme borreliosis.

B. burgdorferi alter its gene expression profile as it cycles between arthropods and mammals, and specific genes help maintain the spirochete in nature. *Ixodes* ticks, while feeding on a mammalian host, deposit a heterogeneous population of *B. burgdorferi* in the dermis, which eventually spread to multiple organs [1]. Selected *B. burgdorferi* genes may be preferentially expressed in different tissue locations [17], however, it is not known if gene products that are preferentially induced in tissues can directly contribute to microbial pathogenesis. Here, we show that both transcripts of the *B. burgdorferi bmpA/B* operon undergo an abrupt up regulation in infected murine joints.

Compared to the arthropod vector, *B. burgdorferi* faces a more complex immune system in mammals. The bacterium, however, is capable of establishing persistent murine infection and can survive in multiple organs [3]. Immune sera from a *B. burgdorferi*-infected host, or specific antisera, when transferred prior to, or at the time of *B. burgdorferi* challenge conferred protection. The same antisera failed to clear *B. burgdorferi* when transferred several days after challenge, despite the capability of injected antisera to modulate disease, such as arthritis [21,22]. These studies clearly compartmentalize the borreliacidal and disease-modifying components in the immune sera.

In nature, *B. burgdorferi* survives in an enzootic cycle where immature ticks acquire the pathogen from wild rodents, and then maintain *B. burgdorferi* and subsequently transmit the spirochetes back to mice [3]. As the tick bite site is primarily located in the murine dermis, the population of *B. burgdorferi* that persistently resides in skin is most likely to complete the tick-mouse-tick life cycle, and should have a selective advantage in nature. The adaptive significance of spirochete's dissemination to deeper host organs [23], such as the joints, where they are unlikely to be acquired by ticks is not clear - although certain sites may afford survival advantages due to protection from host responses, binding ligands or nutrients present in the microenvironment. In fact, our microarray data identified, in addition to specific *bmp* gene, several other *B. burgdorferi* genes are also up regulated in joints and these gene-products might also facilitate spirochete adaptation in a specific microenvironment.

Lyme arthritis is the direct result of a localized host inflammatory response presumably against antigens that are abundant on the surface of the invading spirochete. Joint-induced, surface exposed and highly antigenic *B. burgdorferi* proteins could potentially initiate the complex cascade of host inflammatory response that eventually leading to arthritis [3]. Comparing *B. burgdorferi* transcriptomes from different spirochete-infected murine tissues, we have characterized 2 joint-specific *B. burgdorferi* expression profiles that are selectively expressed in infected joints at different time-points. Our results indicate that *B. burgdorferi* gene expression *in vivo* is predominantly modulated by physiological factors specific for the microenvironment of the particular tissue. This report will facilitate a molecular understanding of joint-spirochete interactions and disease pathogenesis.

In summary, we present here direct evidence for the existence of a dynamic transcriptome of *B. burgdorferi in vivo* and show that differentially up regulated genes, may be critical for spirochete persistence in a specific host environment and influence pathogenesis. This information will contribute to our understanding of adaptive strategies of a pathogen that is highly evolved to survive in multiple host interfaces, and may lead to new strategies to modulate Lyme arthritis.

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