Perspectives Series: Host/Pathogen Interactions

Arthropod- and Host-specific Gene Expression by *Borrelia burgdorferi*

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The threat posed to human health by arthropod-borne bacteria is most vividly demonstrated by the devastating epidemics caused by the plague. The diversity of pathogenic bacteria spread by arthropods is probably underestimated. Recently recognized infectious diseases such as Lyme disease and ehrlichiosis are caused by bacteria transmitted by arthropods. Bacteria which alternate between arthropods and mammals have to survive and thrive in diverse niches. Because of the desire to prevent diseases caused by these organisms, more emphasis had been placed on understanding their life in the vertebrate rather than in the vector. However, understanding how these pathogens develop within their vectors may lead to novel control strategies. The most extensive studies on the development of pathogenic bacteria within arthropods have been done with the agent of plague, *Yersinia pestis*, and its flea vector. Recent studies with *Borrelia burgdorferi*, the Lyme disease spirochete, have also revealed insight into vector-borne transmission of bacteria. The work on *B. burgdorferi* is reviewed here, and when appropriate, contrasted with *Y. pestis*.

In the northeastern United States, the Lyme disease spirochete is maintained in a natural cycle involving rodents and *Ixodes scapularis* ticks. Larval ticks acquire *B. burgdorferi* when they feed on infected mice, and the spirochetes survive through the molts and are present in all subsequent stages (nymphal and adult) of the vector. Mice acquire *B. burgdorferi* from infected nymphs. Studies have focused on events that take place when infected ticks transmit spirochetes to mice. Infected nymphs have several hundred *B. burgdorferi* present extracellularly in the lumen of the gut. When nymphs attach and engorge on mice, the spirochetes multiply to reach densities of $>$ 100,000 bacteria in each tick. The gut is the primary site of *B. burgdorferi* residence and growth. Approximately 48 h into the blood meal, a few bacteria cross the gut epithelium into the hemocoel, invade the salivary glands, and then infect the host (1). Thus, *B. burgdorferi*, which primarily resides in the tick gut, invades the salivary glands only for a brief period during transmission. Similarly, *Y. pestis* resides in the lumen of an arthropod's gut. In the gut of the flea, the bacteria multiply and form a plug that occludes the foregut and prevents successful feeding by the vector. During repeated, futile attempts at feeding by occluded fleas, *Y. pestis* are regurgitated into the mammalian host. Thus, although both *Y. pestis* and *B. burgdorferi* inhabit the gut lumen of their respective vectors, the two organisms use different routes of transmission. *Y. pestis* alters the feeding habits of the vector to increase transmission and eventually kills the flea, while there is no evidence that *B. burgdorferi* alters the behavior or survival of ticks.

How do arthropod-borne bacteria adjust to the different niches they occupy? Several groups have found evidence of *B. burgdorferi* proteins expressed only at certain stages of the life cycle. Outer surface proteins (Osps)[1] A and B are two antigens coded on a bicistronic operon and abundantly expressed on the surface of spirochetes within unfed (flat) ticks. When nymphs feed, the majority of *B. burgdorferi* clear OspA and OspB from their surface and instead express OspC, a protein which is not expressed on spirochetes before tick engorgement (2, 3). Spirochetes that enter the host appear to continue expressing OspC and not OspA or OspB, because mice infected by tick bite rarely develop antibodies to OspA or OspB, while they readily seroconvert to OspC. However, during persistent infection of humans, OspA and OspB must be expressed (at least to some degree) because OspA and OspB antibodies are detectable in some patients with late-stage Lyme disease. Nevertheless, in general OspA and OspB appear to be tick-specific antigens while OspC appears to be expressed in feeding nymphs and in the vertebrate host.

Immunological screening strategies have been developed to identify spirochetal genes that are differentially expressed in the vertebrate host. A *B. burgdorferi* expression library was screened using sera from infected mice and mice immunized with killed cultured spirochetes. Infected serum reflects the antigens that are expressed in the infected host, while the immunized serum represents antigens that are expressed on cultured spirochetes (in vitro) and possibly flat ticks. Antigens that are only recognized by infected sera may reflect genes that are selectively expressed in the mammalian host. The first host-specific gene isolated with this approach was *p21* (4). Messenger RNA for *p21* was readily detected in infected mice and not in cultured spirochetes, confirming the immunologic screening suggestion of in vivo expression. Recently, laboratories have identified more genes (*eppa*, *pG*, *bbk2.10*, and *bmpD*) that appear to be induced in the mammalian host. An interesting feature of many of these genes is that they have homology to *ospE* and *ospF*, two surface proteins of spirochetes coded for by a single operon: both *bbk2.10* and *pG* are homologues of *ospF* while *p21* is an homologue of *ospE*. The *B. burgdorferi ospE/F* homologues appear to comprise a family of genes whose members are expressed at distinct stages of the spirochete's life cycle.

The environmental cues that regulate the expression of *B. burgdorferi* genes are not well understood although recent

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1. *Abbreviation used in this paper:* Osp, outer surface protein.

studies indicate that temperature plays a role. This is logical since arthropods maintain a lower temperature than the vertebrate host. Studies by Stevenson, Schwan, and their colleagues have demonstrated that cultured organisms partially induce *ospC* as well as several proteins of the *ospE* and *ospF* gene family in response to increases in temperature (5). Factors such as components in blood, biochemical changes within unfed and engorging ticks, and the host immune response are also likely to influence *B. burgdorferi* gene expression. In contrast to *B. burgdorferi*, more is known about the signals that regulate *Y. pestis* genes. Some *Y. pestis* genes are induced at 37°C while others are induced or derepressed at 26°C. In addition to temperature, Ca^{2+}, host cell contact, pH, iron, and other small organic and inorganic molecules influence the expression of *Y. pestis* genes (6).

The functions of the differentially expressed *B. burgdorferi* genes are not known. Genes selectively synthesized in the vector may play a role in biosynthesis and nutrient uptake since the single food source of the vector may be very rich in certain nutrients and lacking in others. Ticks digest their blood meal intracellularly by endocytosing the contents of the gut lumen. Spirochetes may need to express genes to prevent their internalization and digestion along with the blood meal. Genes expressed in the vector may also facilitate transmission by permitting the spirochete to invade the gut epithelium and move into the hemocoel and salivary glands. Important topics for future work on *B. burgdorferi* consist of the mechanism of differential gene expression as well as the function of genes expressed at distinct stages.

A remarkable example of how bacterial genes expressed in the vector contribute to transmission involves studies done on the *hms* (hemin storage) locus in *Y. pestis* (7). The *hms* genes are preferentially induced at 26°C, indicating that they may serve an important function in the flea. The genes encoded for by the *hms* locus (*hmsFH*R) permits the storage of large amounts of hemin, heme, and inorganic iron on the outer membrane of the bacillus. Hinnebusch and colleagues reported recently that the blockage of the foregut of infected fleas was dependent on the *hms* locus (7). In syringe-infection experiments *hms*$^-$ mutants were virulent in mice, indicating that this locus was not required for pathogenesis in the host. In contrast, when fleas were infected with *hms*$^-$ mutants, the fleas did not develop blocked foreguts and consequently did not alter their feeding behavior to increase the efficiency of transmission. The exact mechanism by which this locus contributes to blockage is not known; however, in vitro *hms*$^+$ bacteria autoaggregate more easily than *hms*$^-$ bacteria. The aggregated bacteria may form more stable masses that occlude the foregut. This is the first example of a locus in an arthropod-borne bacterium that regulates transmission from the vector to the host.

Once *B. burgdorferi* enters the host, a whole set of new genes may be needed to adapt to the new milieu, combat the host's immune system, and disseminate from the site of deposition (skin) to other sites, including the joint, heart, and central nervous system. In vitro spirochetes bind to decorin, a proteoglycan present on collagen fibers. Decorin binding appears to be mediated by *B. burgdorferi* proteins with apparent molecular masses of 19 and 20 kD (8). In vivo the association with decorin may be important in localizing spirochetes to the skin, which is the site of initial deposition as well as a site of chronic infection. Spirochetes grown in culture also bind to the host derived proteins, plasminogen and urokinase (9). Urokinase cleaves plasminogen to generate active plasmin on the surface of cultured spirochetes. If these events also occur in the host, plasmin which is a serine protease may digest blood clots at the site of tick bite and extracellular matrix components in the skin to facilitate the dissemination of spirochetes. In fact, plasmin-coated organisms displayed an enhanced capacity to penetrate tissue culture cell monolayers and they were more infectious to mice. *B. burgdorferi* proteins with molecular masses of 70 and 30 kD bound to plasminogen. The identity of the larger protein is unknown while the smaller 30-kD protein is OspA. The 70-kD protein which bound 10 times more plasminogen than OspA is more likely to play a role in dissemination in the host since spirochetes entering the host appear to be devoid of OspA.

The gene encoding the *Y. pestis pla* protease is also essential for the effective dissemination of organisms in the host (10). The Pla protease has coagulase and fibrinolytic activity and also cleaves plasminogen to plasmin. The transcription and translation of the *pla* gene is not regulated by temperature although the activity of the enzyme is temperature dependent (11). The fibrinolytic activity of Pla is highest at 37°C while its coagulase activity is highest at 28°C. Since the coagulase activity is highest at the lower temperature, it may function in the vector although the significance of this activity is not clear. The fibrinolytic and plasminogen activating properties of Pla appear to play a prominent role in the dissemination of *Y. pestis* from the site of deposition in the skin of the host. *Y. pestis pla*$^-$ mutants are virulent and produce a systemic infection if introduced by an intravenous route. However, when the *pla*$^-$ mutants were injected subcutaneously to reproduce the normal route of entry, the bacterium produced a localized lesion and did not disseminate to peripheral tissues. Thus, *B. burgdorferi* and *Y. pestis* appear to use two strategies to harness proteolytic activities necessary for dissemination. *B. burgdorferi* binds to host urokinase and plasminogen to produce plasmin while *Y. pestis* produces its own Pla protease which has fibrinolytic and plasminogen activating properties.

The discovery of differential gene expression by *B. burgdorferi* has already had practical ramifications in the area of vaccine development. Mice immunized with OspA are protected from spirochete infection. If spirochetes entering the rodent host are devoid of OspA, why do OspA antibodies protect the host from infection? The clue to this puzzle was the observation that when infected ticks fed upon OspA-immunized mice, OspA antibodies in the blood meal killed spirochetes in the tick gut and subsequently blocked the dissemination of *B. burgdorferi* to the salivary glands of the vector (3). This is the first example of a vaccine undergoing human clinical trials which protects by blocking transmission from the vector. OspA antibody protected mice only when administered within the first 24 h of tick attachment. At later times, spirochetes persisted in the ticks and the mice were infected in spite of the presence of OspA antibodies in the host. These observations are consistent with the hypothesis that as spirochetes clear OspA from their surface, they become resistant to OspA antibody.

Attempts to use *B. burgdorferi* antigens expressed in the host as Lyme vaccines have met with mixed results, presumably because the organism has evolved strategies to protect itself from immune responses against surface antigens expressed in the host. The selection of an antigen selectively expressed in

the vector as a vaccine candidate may be a better choice because in nature the spirochete is unlikely to encounter antibodies against antigens expressed in the vector and may be especially vulnerable to such antibodies as demonstrated by the success of the OspA vaccine in animal models of Lyme disease. The OspA vaccine is being tested currently in humans and the results of these phase III trials are expected soon. Studies on how bacteria adapt to a vector-borne life-style will continue to be a fruitful area of research which may lead to novel interventions for preventing diseases caused by these organisms.

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