Pulling the Trigger on Lyme Arthritis

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The infectious disorders now collectively known as Lyme disease (Lyme borreliosis) began their long ascent to notoriety as a little noticed outbreak of oligoarthritis in Southeastern Connecticut during the mid 1970s [1, 2]. Local physicians mistakenly diagnosed many of these cases, mostly occurring in children, as juvenile rheumatoid arthritis. Two suspicious mothers called the “autoimmune” epidemic to the attention of rheumatologists at nearby Yale University, who made the critical observation that approximately one-quarter of the index case patients had developed a distinctive bull’s eye rash (erythema migrans) similar to one known to be transmitted in Northern Europe by the hard tick *Ixodes ricinus*. Further investigation implicated the deer tick, *Ixodes scapularis*, as the likely arthropod vector [3]. These field studies prompted an intensive microbe hunt resulting in isolation, from ticks [4] and then from a small number of skin, blood, and cerebrospinal fluid specimens [5, 6], of a spirochete subsequently determined to be a new *Borrelia* species [7]. Of the 20 or so species now known to comprise the *Borrelia burgdorferi* sensu lato complex, *Borrelia burgdorferi* sensu stricto (ss), *Borrelia afzelii*, and *Borrelia garinii*, predominate as human pathogens [8]. *B. burgdorferi* ss is the only cause of Lyme disease in the United States. Surprisingly, *B. burgdorferi* ss, the member of the complex with the greatest proclivity to invade joints, has never been isolated from human joints, although spirochetal DNA can be amplified with polymerase chain reaction from synovial fluids. *B. garinii*, a common isolate in Europe, has earned a reputation for neurotropism because of its predilection for invasion of the central nervous system [8]. In nature, Lyme disease spirochetes persist asymptptomatically in rodent reservoirs, such as the white-footed mouse *Peromyscus leucopus* [9]. Because humans are incidental and probably dead-end hosts for the bacterium, illness in humans may be an evolutionarily unintended outcome of the vector’s generalist feeding behavior [10].

Three advances deserve the lion’s share of the credit for the slow but steady accretion of knowledge regarding the spirochete’s enigmatic parasitic strategies. One was the development of a mouse model [9, 11]. Whereas all laboratory strains of inbred mice are susceptible to infection, the propensity to mount a local inflammatory response is highly strain dependent. C3H mice have become the strain of choice because they reliably develop arthritis and carditis, which partially mimic the analogous manifestations in afflicted humans. Of note, no strain of inbred mouse develops erythema migrans or neurologic disease, underscoring the limitations of mice as human surrogates. Studies in severe combined immunodeficiency mice indicate that innate immune responses are the main drivers of tissue damage, whereas adaptive responses, principally antibodies, are required for containment of this extracellular pathogen. Intriguingly, the innate pathways that trigger carditis and arthritis probably differ, based on the finding that cardiac infiltrates consist predominantly of macrophages, whereas those in joints are largely neutrophilic. The second advance was the deciphering of the spirochete’s genetic blueprint, arguably the most complex of all sequenced bacterial genomes [12]. More than 20 genetic elements, including an approximately 1-MB linear chromosome and an assortment of linear and circular plasmids totaling approximately 600 kb, have been identified in the B31 type strain. The recent availability of genomic sequences for numerous strains and genospecies has raised expectations that differences in infectivity, host range, and tissue tropism eventually will be understood at the molecular level [13, 14]. The final advance, the development of robust techniques for genetically manipulating virulent *Borrelia* [15], has enabled...
invasive organisms to negotiate their way from the midgut of a feeding tick to the bite site and then disseminate to target tissues, once they are firmly established in the mammalian host [17–20].

A cardinal difference between B. burgdorferi and gram-negative bacteria, such as Escherichia coli, is the number and variety of lipoproteins known or presumed to adorn the spirochete’s surface [21]. Only a handful of the more than 150 lipoproteins encoded within the borreli genome have proteobacterial orthologs, suggesting that the spirochete has evolved a plethora of novel ploys for interfacing with its mammalian host and arthropod vector. A central tenet of the Lyme disease field is that B. burgdorferi differentially expresses its vast repertoire of surface molecules in order to transit between ticks and mice, disseminate within the mammal following inoculation, and persist within these 2 markedly divergent host milieus [16]. Expression profiles, therefore, provide valuable clues for the identification of lipoproteins likely to function in one host or the other and to contribute to disease production in particular mouse organs.

In an extraordinary series of publications [22–24], the latest in this issue of The Journal [25], Pal and coworkers describe their use of expression analysis of putative surface molecules to select targets for mutagenesis. They begin the current report by showing that expression of bba57, a lipoprotein gene on the linear “minichromosome” lp54, is up-regulated in all tick life stages and in various mouse tissues, including hearts and joints, compared with organisms grown in BSKII medium. This is a remarkable finding given that many characterized borreli lipoproteins are preferentially expressed at a particular stage of the enzootic cycle [16]. After confirming that the BBA75 lipoprotein is exposed on the surface of in vitro–cultivated organisms, they then set about meticulously characterizing the phenotype of a bba57 deletion mutant. Consistent with the expression data, they found that bba57-deficient spirochetes have a complex, possibly pleiotropic phenotype. Ticks microinjected with the mutant were unable to infect mice, whereas mutants inoculated by needle into mice disseminated poorly to various tissues. Unfortunately, the authors did not ascertain whether these results reflect distinct functionalities of BBA57 in ticks and mice or a single defect in survival and/or dissemination at the site of inoculation. Surprisingly, mutant numbers eventually rebounded in all tissues examined, although they remained significantly below wild-type levels in joints. Even more surprising, despite the rebound in numbers, the mutant was unable to induce inflammation in either joints or hearts. By injecting mice with “supraphysiologic” doses of mutants, the authors cleverly circumvented the dissemination defect to achieve normal spirochete burdens in joints; even so, mutant-infected joints displayed much diminished inflammation. Finally, they showed that the pauci-inflammatory property of the mutant could be attributed to a diminished ability to elicit neutrophil-attracting chemokines in joints, with a concomitant impairment in neutrophil recruitment.

Clinical manifestations associated with Lyme disease result from the host’s innate and adaptive responses to the bacterium, rather than from secreted toxicogenic molecules, which Borrelia do not produce [16]. How, then, do Lyme disease spirochetes cause inflammation, which can lead to such vexatious and refractory symptoms in some patients [8]? Less than a decade ago, the answer seemed straightforward. Borrelia contain an abundance of lipoproteins; binding of lipoproteins to CD14 and Toll-like receptor (TLR) 2/TLR1 heterodimers on the surface of phagocytic cells was believed to be all that was required to set the inflammatory ball in motion [11, 16]. There is now a substantial body of evidence, however, to discount the notion of spirochetes as mere bags of lipoproteins. Indeed, recent studies have shown that elicitation of the “full spectrum” of inflammatory cascades in monocytes/macrophages requires internalization of live Borrelia, followed by their degradation within phagosomal compartments, with release not only of lipoproteins but of other microbial products, such as RNA and peptidoglycan [26, 27]. According to this new paradigm, initiation of phagocytosis requires interaction of borreli surface molecules with phagocytic receptors (distinct from CD14 and TLRs) on the surface of innate immune cells. Interaction of spirochetal lipoproteins with integrins on the surface of primary human chondrocytes also has been reported as a mechanism for triggering MyD88-independent inflammatory pathways directly relevant to arthritis development [28].

The article in this issue by Yang et al [25] presents strong in vivo evidence to support this new line of thinking. BBA57 is not an abundant molecule. Although the total lipoprotein contents of wild-type and bba57 mutant Borrelia are essentially identical, their inflammatory capacities within the joint space differ dramatically; bulk proinflammatory activity of lipoproteins simply cannot account for these findings. The authors’ conclusion that BBA57 acts as a molecular trigger for Lyme arthritis by interacting with a cellular receptor within joints seems likely to be correct. The next obvious step, which could have far-reaching therapeutic implications, is to identify the cellular elements in joints that interact with this molecule; in vitro and in vivo methods now exist to accomplish this task.

In the 30 years since the discovery of B. burgdorferi, which virtually coincided with that of human immunodeficiency virus (HIV) [29, 30], probably no infection other than HIV has attracted more attention or become more embroiled in
countroversy [2]. The ultimate challenge will be to devise a translational strategy to establish a definitive link between this new virulence determinant and the pathogenesis of one of the more debilitating consequences of Borrelia infection in humans, the manifestation that kicked off all of the fuss.

Notes

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