


Etiology of the Acrodermatitis Chronica Atrophicans Lesion in Lyme Disease

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Spirochete diversity in acrodermatitis chronica atrophicans lesions in a closely defined central European site was compared to that in the local vector population, in human erythema migrans lesions, and in cerebrospinal fluid by amplifying and sequencing a segment of the gene of outer surface protein A directly from sampled tissues. Borrelia garinii, Borrelia afzelii, and Borrelia burgdorferi acutely infect human skin and invade internal tissues. Only B. afzelii, however, is associated with acrodermatitis chronica atrophicans lesions, persisting chronically where the skin has atrophied.

Lyme disease spirochetes in central Europe appear to be particularly diverse. Seven distinct antigenic variants of outer surface protein A (OspA) [1] and three major sequence variants of 16S rRNA have been distinguished [2]. The general taxon

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Although *B. afzelii* was present in all cultured ACA lesions in patients in western Europe, the same genospecies dominated in cultures isolated from ticks and acute EM lesions [5]. Proof of the genospecies etiology of the ACA lesion, therefore, must await an evaluation of specimens sampled directly from a patient population exposed to diverse spirochete variants.

A definitive etiologic association of ACA requires comparisons based on a site in which the different *Borrelia* genospecies infect the vector population, as in our recent study of neuroborreliosis [3]. Accordingly, we extended this analysis to include ACA lesions in residents of the same site. Spirochete diversity in ACA lesions was compared with that in the vector population, in EM lesions, and in CSF by amplifying and sequencing a segment of the *ospA* gene directly from sampled tissues.

### Materials and Methods

**Patients.** Skin biopsies or CSF samples originated from patients with clinical EM or ACA or with neural manifestations of Lyme disease, respectively. Each had resided in or near Göttingen (in southern Lower Saxony, Germany) for at least 4 months. Subjects were excluded from the study if they reported contact with ticks in other sites during the previous 3 years. None reported recent treatment with antibiotics. Skin biopsies (~3 mm in diameter), sera for laboratory diagnosis, and detailed information regarding clinical manifestations were reported to our department during autumn 1994 and spring and summer 1995. Biopsied tissues were stored in 70% ethanol.

**Ticks.** Ticks were taken from vegetation in three sites in the vicinity of Göttingen. Two (~4 km apart) were in urban parks that contained brushy vegetation, and the third was at the edge of a forest ~3 km from the center of the city.

**DNA extraction, polymerase chain reaction (PCR) amplification, and DNA sequencing.** Total DNA from human skin biopsies was prepared using a QiAamp Tissue kit (Qiagen, Hilden, Germany). Spirochetal DNA was sampled from the midguts of nymphal ticks that were triturated in TE buffer. A specific segment of the spirochetal *ospA* gene was amplified by PCR [3, 8]. For comparison, a CSF sample and a skin biopsy that contained *B. burgdorferi* as well as 2 samples free of spirochetes accompanied each series of amplification. DNA was extracted, reaction vials were prepared for amplification, and products were electrophoresed in separate laboratories. Amplified DNA fragments were sequenced by the dideoxynucleotide chain-termination method on an ABI 373 DNA-sequencer (Applied Biosystems, Weiterstadt, Germany).

**Relationships between Borrelia genospecies in groups of samples were compared by χ² analysis. First we tested for heterogeneity between different kinds of samples with a 2 × 3 analysis. We then compared the distribution of genospecies in each kind of sample to that in the vector population with a series of 2 × 2 analyses.**

### Results

To determine the genospecies diversity of spirochetes present in Göttingen ticks, a segment of the *ospA* gene was amplified and sequenced from each midgut sample. Of 165 ticks, 52 were infected with the agent of Lyme disease. Five of these ticks were infected by more than one genospecies. Of the 47 ticks infected by only one kind of spirochete, *B. garinii* proved to be most prevalent, *B. afzelii* was next, and *B. burgdorferi* s.s. was least abundant (table 1). This ratio of genospecies prevalence in local vector ticks approached 10:5:1, respectively, and did not vary between sites.

We compared the distribution of the *Borrelia* genospecies found in human residents of the study site who suffered from Lyme disease with that in ticks. These data included those that we have reported previously [3]. Only 1 tissue sample was taken from each of the 35 subjects. The prevalence of genospecies in acute EM lesions and in CSF did not differ significantly from that in the vector population (table 1). The distribution of genospecies infecting chronic ACA lesions, however, differed significantly from that in the vector population and from that in EM or CSF samples. Although all three genospecies may be present in acute dermal or CNS lesions, only *B. afzelii* was associated with persistent ACA lesions.

### Discussion

Persuasive evidence of the genospecies etiology of the ACA lesion should derive from comparisons of the diversity of pathogens in lesions with that in the local vector population. Indeed, we have demonstrated a skewed relationship; *B. afzelii* found in all ACA lesions that we studied was present in less than half of the sympatric infected ticks that we examined. In contrast, the distribution of spirochetal genospecies in acutely infected skin parallels that in vector ticks, and we have previously reported that CSF samples contain a similarly natural array of spirochete genospecies [3]. This implies that spirochetes of all three genospecies infect human skin and invade internal tissues, but that only *B. afzelii* remains in skin chronically.

*B. afzelii* has previously been associated with ACA lesions [1, 5–7]. Spirochetes of this genospecies were found in virtu-
ally all samples biopsied from EM and ACA lesions in patients referred to an Amsterdam hospital as well as in ticks from two remote sites in the Netherlands [5]. A subsequent survey of ticks from various parts of that country, however, found that few contained B. afzelii [9]. The 10:1:15 ratio of the three genospecies (B. garinii, B. afzelii, B. burgdorferi) in this second Dutch study [9] differed from that (10:5:1) in our sample of Göttingen ticks and from the 8:1:4 ratio in ticks collected throughout central Europe [1]. We know of genospecies analyses in which B. afzelii was present in all but 1 of 16 isolates derived from ACA lesions in European residents [1, 6, 7]. These analyses, therefore, associate this genospecies with ACA lesions but fail to provide a reference point based on the prevalence of spirochete genospecies in the local vector population.

Although B. afzelii is associated largely with the skin [1, 5, 7], spirochetes of this genospecies may infect other human tissues. Patients with aseptic meningitis or acute peripheral facial palsy are infected by B. afzelii as frequently as are vector ticks [3]. We now demonstrate that the distribution of spirochetes in acute EM lesions similarly reflects that in the population of infecting ticks. Although vector ticks deposit each of the spirochete genospecies directly into the skin, B. garinii and B. burgdorferi may fail to persist in this superficial tissue.

Other investigators have relied on cultured isolates of Lyme disease spirochetes for their study samples [1, 5-7]. As in our previous study [3], we amplified spirochete DNA from the primary sample. Such direct analysis avoids several potential pitfalls. The use of antibiotics in the culture medium may disproportionately select against certain genospecies; B. garinii, in particular, seems not to thrive in antibiotic-containing culture medium [5]. Then too, spirochete populations may interact such that one genospecies in a mixed population would be lost during culture. Our PCR procedure, however, detects every kind of spirochete in a mixture even when those of another genospecies are five times more abundant [3]. Direct amplification of primary samples averts bias due to antibiotic selection or interpopulational interactions.

It seems curious that so few ticks and no clinical specimens in our study contained more than a single genospecies. If these pathogens were distributed randomly in nature and if reservoir rodents were subject to frequent reinfection, we would anticipate that far more ticks would be multiply than singly infected. The 10:5:1 genospecies ratio that we observed is, therefore, inconsistent with panmixia. Indeed, only B. afzelii could be cultured from the ear pinnae of various Swiss rodents [10]. Whether this association derives from natural or artificial selection remains unknown. However it may well be that the environment of the various Lyme disease spirochetes is somehow partitioned.

Our observations are consistent with the general concept that ACA seems not to occur in the Americas. Although the B. afzelii genospecies may cause various internal as well as skin lesions, the ACA lesion appears to result exclusively from infection by this variant of the Lyme disease spirochete. We conclude that B. afzelii is the etiologic agent of the ACA lesion in Lyme disease.

References