Borrelia burgdorferi genospecies in humans and ticks in the Alpe Adria region

M. Cinco

SUMMARY

In this paper we report on the genospecies prevalence of *Borrelia burgdorferi* sensu stricto we found in human Lyme borreliosis and in the tick vector *Ixodes ricinus* in two distinct but restricted areas of the Alpe Adria region, the Karst and Belluno territory. Different rates of genospecies prevalence were found in the two areas studied, in humans as well as in ticks, with a net predominance of *Borrelia afzelii* in human infection in the Belluno area.

KEY WORDS

Borrelia burgdorferi, Borrelia afzelii, isolation of strains, ticks, detection of DNA, Ixodes ricinus, epidemiology, RFLP profiles, genospecies

Introduction

Lyme borreliosis (LB) is endemic in North-Eastern Italy, affecting humans mainly in the Friuli Venezia Giulia and Veneto regions. About 50 cases per year have been recorded at the Hospital of Cattinara, Trieste (Department of Dermatology), and 119 patient with LB have been recorded at the Hospital of Belluno during the 1993-98 period.

Investigations on the presence of *Borrelia burgdorferi (Bb)* sensu lato, the etiological agent of LB in Italy have been carried out mainly in the Karst territory by isolating strains and by detecting *Borrelia* DNA in the tick vector *I. ricinus* by PCR (1,2), leading to the identification of the circulating genospecies and the risk locations. Since the prevalence of the circulating genospecies of *Bb* is important for epidemiological reasons and for basic knowledge on the biology of the organism, both in humans and ticks, we report here the comparative data obtained from the identification of the *Bb* strains isolated from patients with genospecies found in ticks, in the Karst and Belluno areas.

Methods

Clinical samples and isolation of Bb

Samples of skin biopsies, blood, heart biopsies, or cerebrospinal fluid were taken from patients with various manifestations of LB and put into BSK medium containing 5% normal rabbit serum. All specimens were incubated at 32°C for at least two months, and samples of each culture were examined for spirochetes weekly

by dark-field microscopy.

Classification of isolated Bb strains

Bb strains were classified by PCR-RFLP analysis of the *rrf*A-*rrl*B intergenic spacer (1) and by chromosomal analysis with pulse field electrophoresis (PFGE). This latter method was performed by Dr. Lorenzo Ciceroni at Istituto Superiore di Sanità (Rome). Genomic DNAs were prepared as described by Taylor et al. (2) with slight modifications.

Detection of B. burgdorferi DNA in Ixodes ricinus.

Ticks collected in different areas of the Karst and Belluno territory were processed for DNA extraction and Borrelia DNA detection by PCR. Different levels of PCR sensitivity and specificity were used in our Laboratory, and finally we chose the technique of Reverse Blot Line Hybridization as reported (3-7), which allowed us to individuate the genospecies of the infecting Borreliae. Mixed infections were often reported.

Results and discussion

The samples of the patients from the Karst area were from erythema migrans, disseminated erythema migrans, arthritis, and myocarditis: the isolated strains belonged to all three *Bb* genospecies known to be human pathogens, *Borrelia garinii, Borrelia burgdorferi* sensu stricto and *Borrelia afzelii*, with a prevalence of 40%, 30% and 30%, respectively. Strains isolated from erythema migrans patients of the Belluno area predominantly belonged to the *Borrelia afzelii* genospecies. The RFLP profiles were obtained after MSEI digestion of the amplicon. The majority of the profiles were *B. afzelii* (85%). Only two strains (5%) belonged to the *B. garinii* genospecies and two strains (5%) were *B.burgdorferi* sensu stricto. These data were confirmed by the PFGE analysis performed at the Istituto Superiore di Sanità, Rome.

The prevalence of *Borrelia* genospecies in *I. ricinus* was determined by PCR and Reverse Blot Line Hybridization. Four genospecies were detected, *B. afzelii, B. garinii, B. burgdorferi* sensu stricto, and *B. valaisiana*, very often in combination; most prevalent was *B. garinii* alone (30%), followed by the association between *B. garinii* and *B. burgdorferi* sensu stricto (32%); less prevalent were other combinations and only 2% and 4% of the ticks were infected with either *B. afzelii* or *B. valaisiana.*

In the Belluno area the genospecies *B. burgdorferi* s.s., *B. valaisiana* and *B. afzelii* were detected in ticks (Grazioli et al., personal communication), but the rate of infection of *B. afzelii* was not as high as found in humans.

In conclusion, different rates of genospecies circulation exist in the two areas examined. Of note is the higher prevalence of isolates belonging to the genospecies *B. afzelii* among humans. Similar epidemiological findings have been reported from Slovenia (8). The dominant prevalence of *B.afzelii* in humans in the Belluno area may be due to the fact that the isolates were obtained from patients with erythema migrans; there is general agreement that skin manifestations are most often caused by this genospecies. Moreover, our data suggest that genospecies prevalence in ticks does not reflect the genospecies prevalence in infected humans. It may be hypothesised that this finding is due to a different selective pressure performed by the different animal hosts and ticks on *Borrelia* strains having which have a different complement resistance. It has been demonstrated that strains of *B. afzelii* are resistant to human complement (9), but many strains of *B. garinii* are not. Therefore this latter genospecies is more favorite to maintain the infection in ticks than in mammalian hosts.

Acknowledgments

We thank C. Zasio and G. Bertiato, Microbiology Laboratory, Hospital of Belluno, for isolation and sending of *B. burgdorferi* strains from Belluno.

REFERENCES

1. Postic D, Edlinger CR, Grimont F et al. Two genomic species in Borrelia burgdorferi. Res Microbiol 1990; 141: 465-75.

2. Taylor A, Barbour AG, Thomas DD. Pulsed-field gel electrophoretic analysis of leptospiral DNA. Infect Immun 1991; 59: 323-9.

3. Cinco M, Padovan D, Murgia R et al. Rate of infection of I. ricinus ticks with Borrelia burgdorferi sensu stricto, Borrelia garinii, Borrelia afzelii and group VS 116 in an endemic focus of Lyme disease in Italy. Eur J Microbiob Infect Dis 1998; 17: 90-4.

4. Frusteri L, Padovan D, Murgia R et al. Studies on natural infection of Ixodes ricinus (Acarina: Ixodidae) in Italy: Report on multiple microorganisms coinfection (B.burgdorferi and Ehrlichia spp.).Alpe

Adria Microbiology Journal, 1998; 2: 123-31.

5. Cinco M. La borreliosi di Lyme. Monografia, Collana Caleidoscopio, 1988; N° 122.

6. Ciceroni L, Ciarrocchi S, Ciervo A et al. Isolation and characterization of Borrelia burgdorferi sensu lato strains in an area of Italy endemic for Lyme Borreliosis J Clin Microbiol 2001; 6: 2254-60.

7. Cinco M, Padovan D, Murgia R et al. Prevalence of Borrelia burgdorferi Infection in Ixodes ricinus in Central Italy. Eur J Clin Microbiol Infect. Dis. 1998; 17: 131-6.

8. Picken R, Cheng Y, Strle F, et al. Molecular characterization of Borrelia burgdorferi sensu lato from Slovenia revealing significant differences between tick and human isolates. Eur J Clin Microbiol Infect Dis. 1996; 15: 313-23.

9. Breitner-Ruddok S, Wurzner R, Schulze J et al. Heterogeneity in the complement-dependent bacteriolysis within the species of Borrelia burgdorferi. Med Microbiol Immunol 1997;185; 253-60.

A U T H O R 'S A D D R E S S *Marina Cinco, PhD, Department of Biochemistry Microbiology Section, Lab Spirochetae, Via Fleming 22, Università di Trieste, 34 127 Trieste, Italy*