Borrelia burgdorferi in rodents (Apodemus flavicollis and A. sylvaticus): Duration and enhancement of infectivity for Ixodes ricinus ticks

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Abstract. Ixodes ricinus is an important vector of Borrelia burgdorferi in Europe, and small rodents (Apodemus flavicollis, A. sylvaticus and Clethrionomys glareolus) are important sources for infecting ticks. In this study, we examined their reservoir role by studying the duration of their infectivity for ticks. A. flavicollis and A. sylvaticus mice captured in nature were exposed to uninfected I. ricinus larvae at different times after their capture: 10 days, and 2, 7, 11, 14 and 40 months. Ticks were examined for spirochaetes after moulting using direct immunofluorescence. All animals remained infective for ticks their life long but the efficiency of transmission from hosts to ticks varied from one individual to the other, presenting a three-fold variation (26.5% to 81.4%). Rodents continously exposed to successive infestations by larval I. ricinus ticks over a period of one month showed an enhancement of infectivity for larval ticks during this period.

Key words: Apodemus sp., Borrelia burgdorferi, Infectivity to tick, Ixodes ricinus

Introduction

Lyme borreliosis in humans and animals is a multisystemic disease caused by Borrelia burgdorferi Johnson, Schmid, Hyde, Steigenwalt et Brenner, a spirochaete transmitted by ticks. In Europe, the main vector is Ixodes ricinus Linné, and small rodents, Apodemus flavicollis Melchior, A. sylvaticus Linné and Clethrionomys glareolus Schreber, are the most important hosts for infecting I. ricinus ticks [1–5]. The duration of infectivity of these hosts for ticks remains unknown, although some studies suggest prolonged persistence of B. burgdorferi in the rodent Peromyscus leucopus (Rafinesque), the most efficient source for infecting Ixodes dammini Spielman, Clifford, Piesman et Corwin (at present I. scapularis Say [6]) in the USA and in laboratory reared mice [7–9]. We now show that field-collected A. flavicollis and A. sylvaticus are infective for I. ricinus larvae during their whole life and that this infectivity is increased in response to tick infestations.

Materials and methods

Three A. flavicollis (G209, G219, G220) and two A. sylvaticus (G149, G210) were captured in October 1988 in a Lyme disease focus, the Staatswald (Berne, Switzerland) [3] and maintained in the laboratory.

To determine duration and degree of infectivity of the small mammals, xenodiagnosis (xe) [10] was used: clean ticks were allowed to feed to repletion on the rodents. After moulting the ticks were dissected and examined for the presence of borreliae. All animals remained infective for ticks their life long but the efficiency of transmission from hosts to ticks varied from one individual to the other, presenting a three-fold variation (26.5% to 81.4%). Rodents continously exposed to successive infestations by larval I. ricinus ticks over a period of one month showed an enhancement of infectivity for larval ticks during this period.

During tick infestation, all mice were kept individually in cages held over trays of water. Larvae were always put on the head of the mouse, where most of the larvae are located on captured animals, and a collar was placed around the neck to prevent grooming. Larvae engorging on the infected rodents were collected as they detached, placed into vials, and stored in humid chambers at about 95% humidity and 22 °C until nymphs emerged.

The prevalence of infection in I. ricinus nymphs was determined using direct immunofluorescence (DIF). Ticks were smeared in toto in a small drop of...
PBS pH 7.4, on a glass microscope slide, air dried and fixed in an acetone bath for 10 min. Slides were treated as described previously [11] with a fluorescein isothiocyanate-conjugated polyclonal antibody prepared by hyperimmunization of New Zealand white rabbits using the American strain B31. They were examined at ×400 magnification by fluorescent microscopy. The density of spirochaetes within I. ricinus was evaluated by counting the spirochaetes in each tick. Five infection levels were determined: (a) 1 spirochaete per tick, (b) 2–10 spirochaetes per tick, (c) 11–50 spirochaetes per tick, (d) 51–100 spirochaetes per tick, and (e) > 100 spirochaetes per tick.

Monoclonal antibodies H5332 (kindly provided by Dr Alan Barbour) specific for the OspA of B. burgdorferi strain B31 [12] were used to characterize the spirochaetes present in some of the ticks fed on the small mammals. B. burgdorferi was isolated from I. ricinus nymphs fed as larvae on the field-collected mice. Nymph midguts were incubated individually in BSKII medium [13]. All isolates were subjected to SDS-PAGE as described previously [14]. Briefly, whole cells were suspended in 15 µl distilled water and resuspended in sample buffer to give a protein concentration of 30 µg/µl. The pH of the separating gel was 8.8 and the acrylamide concentration was 12.5%. The resolving gel was 120 mm high, 140 mm in width, and 0.75 mm thick.

Linear logistic regression analysis for binary response data [15] by the method of maximal likelihood of SAS software was employed to analyse the increase of transmission of B. burgdorferi from infected rodents to uninfected ticks during the successive infestations corresponding to xen. The binary response for each tick is one when transmission of spirochaetes from the host to the tick occurs and zero when such a transmission does not occur. \( p \) is the probability of B. burgdorferi transmission from the mouse \( k \) to the ticks after \( z \) previous infestations during xen. The chosen linear model was the following:

\[
\text{logit}(p) = \log\left(\frac{p}{1 - p}\right) = \alpha_k + \beta \cdot z \quad 1 \leq k \leq 5
\]

\( \alpha_k \) are 5 intercept parameters, one for each mouse and they determine the probability of transmission without previous infestations; \( \alpha_0 \) is the start value of logit(\( p \)). \( \beta \), the slope parameter (the same for all mice), determines the variation of the B. burgdorferi transmission proportion for the successive infestations of xen.

Results

Spirochaetes infecting I. ricinus that had fed on animals G149, G210 and G220 reacted with the monoclonal antibody H5332 whereas spirochaetes present in ticks that had engorged on mice G209 and G219 did not react with this monoclonal antibody. Borreliae were isolated successfully from I. ricinus nymphs of 3 of the 5 rodents (G149, G210, G220). Migration profiles of whole-cell lysates of the isolates were heterogeneous when examined by SDS-PAGE and stained by Coomassie blue (Figure 1). Two isolates (from mice G149 and G210) presented a 22 kDa protein whereas the third one (from mouse G220) did not express this protein and differences in the molecular weight and the intensity of Osp proteins (OspA and OspB) were observed between the isolates.

All animals infected ticks during their life in the laboratory (40 months (G149, G210) and 14 months

![Figure 1. Representative SDS-PAGE analysis of Borrelia burgdorferi strains stained by Coomassie blue isolated from 3 Ixodes ricinus ticks fed on 3 different rodents. Lane 1: mouse G149; Lane 2: mouse G210; lane 3: mouse G220.](image-url)