ISOLATION AND ANTIGENIC VARIABILITY OF BORRELIA BURGDORFERI

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SUMMARY

Organisms of the species *B. burgdorferi sensu lato* are isolated from ticks, mainly of the genus *Ixodes*, and hosts- birds and mammals- by different sampling and using BSK medium. Isolation for diagnostic purposes, is the best demonstration of the etiology of the infection, but it is not always successful due to the paucity of spirochetes in the body.

Strains of *Borrelia burgdorferi* undergo antigenic variations, which affect Osp main proteins and also LOS. Some of these antigenic changes studied in the so-called "escape mutants" on a molecular point of view, showed to be due to intraplasmid recombinations, at the *Osp* gene sequences. These mutations can play an important role during the *Borrelia* infection "in vivo".

KEY WORDS

Lyme borreliosis, Borrelia burgdorferi, isolation, antigenic variability, Osp main proteins, LOS, "escape mutants"

INTRODUCTION

Unlike some members of the genus *Borrelia*, spirochetes of the species *Borrelia burgdorferi* are currently cultivable in the liquid medium named BSK-II (1) and, with some difficulties in the same medium solidified by the addition of agar (2). This fact has made possible the isolation of the organisms from their natural sources which are animal hosts and vectors.

Whatever the source of Borreliae, the biologic material has to be transferred into the appropriate medium, the method and time of processing depend on the origin of the sample.

The sample is inoculated into the liquid medium and serial passages are made - at week intervals- from the original tube. The incubation is performed

at 34°C and the cultures are monitored weekly by darkfield microscopy for the presence of spirochetes. The reduplication time of *Borrelia burgdorferi (Bb)* is very long and moreover the adaptation of the organism to the artificial medium can cause a relevant delay in the growth of the isolate: for this reason the cultures are kept under microaerophilic conditions, for at least one month, before discarding it as negative.

Isolation from animals

B. burgdorferi seems to be widespread in the mammal kingdom, the invertebrates and birds are also involved. The isolation of borreliae is not easily performed from whichever host; in fact attempts of isolation of borreliae from large domestic animals and wild ungulates were unsuccessful, in spite of the demonstration of the organism in the tissues (3). 0n the other hand isolation, from small rodents is easily performed, because these animals are natural reservoirs and spirochaetemia occurs often during the course of the *Borrelia* infection; indeed the infection is long lasting. Isolation of *Bb*. from rodents is mostly successful from urinary bladder (4), but can be easily done also by ear punch biopsy with 95% of probability; this latter procedure is very useful for field and laboratory experiments (5).

Bb can be isolated also from the liver of birds which appeared to be infested by Ixodides; this was the first proof that *Bb* is able to infect also birds (6)

Isolation from ticks

Ticks, mainly of the genus *Ixodes*, represent the main vector of Bb which they harbour in the midgut. The processing of ticks for isolation of *Bb* occurs with caution to avoid contamination. After immersion for few seconds in 70% isopropanol ticks are dissected and the midgut inoculated into selective BSK medium to prevent residual contaminants. Common antibacterials added to BSK medium include Kanamycin, 5-fluoruracil (7) and fosphomycin.

Isolation from humans

The isolation of *Bb* from man represents the strongest evidence of the infection and confirm without doubts the diagnosis of Lyme disease. Nevertheless only few cultures from definite cases of Lyme borreliosis yield spirochetes, because of the paucity of these organisms in tissues and body fluids. Borreliae can be isolated from blood, cerebrospinal fluid (CSF) skin (ECM), or joint fluid, depending on the stage of the disease. During early localized or disseminated borreliosis, spirochetes can be isolated from ECM: recently it has been reported that skin biopsies taken from the peripheral border of the lesions can give 86 % of positive culture compared to 57 % of positivity for specimens taken from perilesional areas (8); thus ECM represents the site from which the probability of successful isolation is the highest. Less frequent is the recovery of Borreliae from blood and CSF. We recently obtained positive cultures from myocardium biopsies of two cases of acute carditis which developed in the absence of cutaneous lesions (Cinco et al, in press). This stresses the importance of direct isolation in the early stage of the illness.

Antigenic variations in Borreliae burgdorferi.

The antigenic heterogeneity of *Bb* is a phenomenon recognized by all the investigators: in fact within *B. burgdorferi in sensu lato* different expressions of the main outer surface proteins OspA, OspB and OspC were demonstrated. The analysis of the major OspA complex by Wilske (9) by employing monoclonal antibodies directed against selected OspA domains has led to the individuation of seven serotypes: accordingly serotype 1 belongs to genosp. *Borrelia sensu stricto;* serotype 2 to Borrelia *group VS461* and serotypes 3-7 to *Borrelia garinii.* This last genospecies shares more polymorphism concerning OspA and also OspC.

Beside the interstrains antigenic heterogeneity *B. burgdorferi* can show antigenic variability also within the strain. During the *vivo/vitro* transfer and after about 11-25 subcultures, Borreliae undergo antigenic modifications which affect as surface proteins as LOS. In fact (10) during seral *in vitro* passages strains loose their OspB component and increase the expression of p20 and LOS: in a recent investigation (11) we found that the 10th passage of a wild derivative did not expressed p39 as the first isolate and, after the selective pressure of homologous antibodies, looses the LOS component (12).

Beside these spontaneous antigenic variations and clonal polymorphism detected *in vitro*, there is also

evidence of antigen changes *in vivo:* in fact during the persistent infection experimentally induced in *Peroniscus leucopus* (13) the *Borrelia* reisolates showed a stronger reactivity towards p39. Recently Hu (14) demonstrated that 7 out of 9 Borrelia strains, reisolates from artificially infected *I. ricinus* expressed different patterns of immunodominant proteins. Only recently a molecular approach to explain such phenotypic variations was carried on following the classic methods of genetics. The group of P. Rosa (15, 16) obtained "escape mutants" under the selective pressure of monoclonal anti OspA and OspB antibodies. These variants showed different type of mutations like complete loss of OspA and OspB and formation of single chimeric OspA/B. Further analysis of the *Osp* loci on several *Borrelia* strains expressing different Osp phenotypes demonstrated that the variations were true mutations due to homologous intraplasmid recombination which both delete *Osp* gene sequences and chimeric gene fusions. Other molecular rearrangements were non sense mutations and sequence divergence.

These data are very important: though the frequency of spontaneous recombination is not known, it is a relevant source of escape variants under the selective pressure of the host immune defenses, contributing to the spirochete persistence. Further the potential for antigenic variation *in vitro*, though not so frequent as the antigenic variations which occur in Borreliae of relapsing fever at the level of Vmp proteins by multiphasic antigenic variation, can have implication for the utility of OspA and OspB as diagnostic or vaccine candidate for Lyme disease.

REFERENCES

1. Barbour AG. Isolation and cultivation of Lyme disease spirochetes. Yale J Biol Med 1984; 57: 521-525.

2. Kurtti TJ, Munderloch UG, Johnson RC, et al. Colony formation and morphology in Borrelia burgdorferi. J Clin Microbiol 1987: 25: 2054-2058.

3. Durey PH. Visceral histopathology in Lyme borreliosis: new observations. Zentralbl Bakt Suppl 1989; 18: 116-125.

4. Schwan TG, Burgdorfer W, Schrumpf ME, Karstens RH. The urinary bladder, a consistent source of Borrelia burgdorferi in experimentally infected white-footed mice (Peroniscus leucopus). J Clin Microbiol 1988; 26: 893-895.

5. Sinsky RJ, Piesman J. Ear punch biopsy method for detection and Isolation of Borrelia burgdorferi from rodents. J Clin Microbiol 1989: 27: 1723-1727.

6. Anderson JF, Johnson RC, Magnarelli LA, Hyde F. Involvement of birds in the Epidemiology of the Lyme disease agent Borrelia burgdorferi Infect Immun. 1986. 51: 394-396.

7. Johnson SE. Klein GC. Schmid GP, et al. Lyme disease: a selective medium for isolation of the suspected etiological agent, a Spirochete. J Clin Microbiol 1984: 19: 81-82.

8. Berger BW. Johnson RC, Kodner C, Coleman L. Cultivation of Borrelia burgdorferi from erythema migrans lesions and perilesional skin. J Clin Microbiol 1992; 30: 359-361.

9. Wilske B, Preac-Mursic V, Jauris S, Hofmann A, Pradel I, Soutschek E, Schwab E, Will G, Wanner G. Immunological and molecular polymorphisms of OspC, an immunodominant major outer surface protein of Borrelia burgdorferi. Infect Immun. 1993; 61: 2182-2191.

10. Schwan TG, Burgdorfer W. Garon, CF. Changes in infectivity and plasmid profile of the Lyme disease Spirochete, Borrelia burgdorferi, as a result of in vitro cultivation. Infect Immun. 1988; 56: 1831-1836.

11. Cinco M, De Giovannini R. Phenotypic variations during in vitro cultivation of a fresh isolate of Borrelia burgdorferi. In press.

12. Cinco M. Selection of a Borrelia burgdorferi antigenic variant by cultivation in the presence of increasing amounts of homologous immune serum. FEMS Microbiol Lett. 1992; 15-18.

13. Schwan TG. Karstens RH, Schrumpf ME, Simpson WJ. Changes in antigenic reactivity of Borrelia burgdorferi, the Lyme disease spirochete during persistent infection in mice. Can J Microbiol

1991;37:450-454.

14. Hu CM, Gern L, Aeschlimann A. Changes in the protein profile and antigenicity of different Borrelia burgdorferi strains after reintroduction to I. ricinus ticks. Parasite Immunol 1992; 14: 415-427.

15. Sadziene A, Rosa P, Thompson PA, Hogan DM, Barbour AG. Antibody resistant mutants of Borrelia burgdorferi: in vitro selection and characterization. The J Exp Med 1992; 176: 799-809.

16. Rosa P, Schwan TG, Hogan D. Recombination between genes encoding major outer surface proteins A and B of Borreliae burgdorferi. Mol Microbiol 1992; 6: 3031-3040.

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