Scientific Works. Series C. Veterinary Medicine. Vol. LXX (1), 2024
ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295

DEVELOPMENT OF A MURINE MODEL OF NEUROBORRELIOSIS INDUCED BY HUMAN *BORRELIA* STRAIN

Cristin COMAN[1, 2, 3], Diana-Larisa ANCUȚA[1, 2], Fabiola-Margareta IONIȚĂ[1, 2], Ioana Violeta CARACOTI[4], Costin CARACOTI[4], Andrei Alexandru MUNTEAN[1, 4], Mircea Ioan POPA[1, 4]

[1]"Cantacuzino" National Institute for Medico-Military Research and Development, 103 Splaiul Independentei, District 5, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, 105 Splaiul Independenței, District 5, Bucharest, Romania
[3]Fundeni Clinical Institute, Center of Excellence in Translational Medicine, 258 Fundeni Road, District 2, Bucharest, Romania
[4]"Carol Davila" University of Medicine and Pharmacy, 8 Eroii Sanitari Blvd, District 6, Bucharest, Romania

Corresponding author email: comancristin@yahoo.com

Abstract

The genus Borrelia is represented by spirochetes that, once entered the human or animal body, can migrate to different organs, including the central nervous system. The aim of the study was to develop a murine neuroborreliosis model by inoculating a strain of Borrelia burgdorferi (Bb). 2 studies were conducted using BALB/c and CD1 mice strains, female, 8 weeks old, 22 grams at baseline. The selected animals were inoculated with Bb (10^6 spirochetes/mL), depending on the protocol, intradermally (100µL/animal) or intracerebrally (10µL/animal). For the evaluation of the neuroborreliosis development, endpoints were established at 1, 3, 6, 8 and 13 weeks post-infection, when samples of dura mater, brain, ear, heart, knee joint and bladder were collected, and maintained in culture medium specific for the growth of Borrelia. Recovery of Bb was possible from samples harvested 7 days post-infection from animals inoculated both intradermally and intracerebrally. Through the experiments we have developed a model of neuroborreliosis in two mice strains that can be useful for the study of the development of new therapeutic approaches.

***Key words**: Borrelia burgdorferi, intracerebral, intradermal, neuroborreliosis, mouse.*

INTRODUCTION

Borreliosis or Lyme disease is caused by spirochetes belonging to the genus *Borrelia burgdorferi* (B.b.): B.b. sensu strictu, *B. garinii* and *B. afzelii* (Baranton, 1992). They are transmitted to humans and animals via ticks of the genus Ixodes (Zawada, 2020).
The history of borreliosis dates back to 1977, when it was clinically described as an infectious disease (Steere, 1977). The characteristic feature of the disease is a rash called erythema migrans complemented by signs of influenza, arthritis, cardiac or neurological disorders (van Dam, 1993; Shapiro, 2020).
The natural reservoir of B.b. is the white-footed mouse *Peromyscus leucopus* (Zawada, 2020).
In the process of transmitting borrelia into the human or animal body, changes in spirochete gene expression occur whereby they adapt and replicate. The motility and chemotaxis characteristics of borrelia enable systemic dissemination of infection by adhesion to the vascular wall or extravascular tissue, and the immune response generated determines the clinical expression of the disease. In humans, Lyme disease can persist for a long time before diagnosis and treatment, and in experimental infections in mice, borreliosis can persist for life (Rudolf, 2021).
The mouse model is often used to investigate the pathophysiology of human disease, including the study of Lyme disease. C3H/HN mice, have been shown to be most susceptible to borrelia infection, expressed clinically by inflammatory carditis and arthritic manifestations (Barthold, 1993). In these animals, the mode of induction of the disease that allows survival but especially migration and multiplication of spirochetes is intradermal inoculation, by this approach also

reproducing the natural conditions of infection (Kern, 2015). The skin is the organ that plays an essential role in the biology of borreliosis. At this level, borrelia cause chronic infection and from this point, migrate to other organs (Barthold, 1993).

On the other hand, there are reported data in the literature showing that borrelia could be recovered from the skin of mice almost a year after infection (Grillon, 2017). Several routes of disease induction have been tried over time (intraperitoneal, intradermal, intracerebral, subcutaneous, intravenous or by direct tick-animal contact) and it has been concluded that mice express disease when inoculated intradermally (Barthold, 1991). The strain of mouse appears to influence how the disease manifests, thus C3H mice develop severe joint and heart disease, C57Bl6 and BALB/c, mild forms of the same disease, and in SCID mice, the disease is highly aggressive, with arthritis and carditis reaching a maximum severity within 60 days of inoculation (Barthold, 1992).

Although the murine model is successfully used to study arthritis and carditis following Lyme disease, there is limited data in the literature focusing on the kinetics of B.b. in the nervous system (Garcia-Monco, 2013). For this reason, the aim of our study was to develop a mouse model of neuroborreliosis that would contribute to our understanding of host-pathogen interactions in the central nervous system (CNS).

MATERIALS AND METHODS

Ethics statement

The animal studies were approved by the Ethics Committee of the "Cantacuzino" National Institute for Medico-Military Research and Development (CI) and by the competent authority (Sanitary Veterinary and Food Safety Directorate Bucharest). The experiments were also carried out in accordance with EU Directive 63/2010 on the protection of animals used for scientific purposes.

Animals

Two mouse strains were used: BALB/c and CD1, derived from the CI Specified Pathogen-Free Animal Facility. Selection of animals at the start of the experiments was based on age (8 weeks), sex (females) and weight (approx. 22 grams). Mice were housed at the Experimental Medicine and Translational Research Platform facility in individually ventilated cages with 12-hour light-dark cycles. Animals were housed in groups of 5 (experiment no. 1) and 6 (experiment no. 2), respectively, and received water and food *ad libitum.*

Borrelia burgdorferi strain

The B.b. strain was provided by the Institute of Neuroimmunology of Kosice, Slovakia, and was isolated from the cerebrospinal fluid of a human patient. The strain processed by introducing a Green Fluorescence Protein (GFP) plasmid and a gentamicin-resistant gene. B.b. revitalisation was performed in the CI microbiology laboratory and for each experiment a low passage number (<6) Borrelia GFP strain was used, which was grown in BSK-H medium supplemented with 6% rabbit serum and 100 µg/1000 µl Gentamycin until the spirochete concentration was sufficient for use in the experimental infection. Inoculum was measured using the counting chamber of the phase contrast microscope and the concentration of borrelia used was set at 10^6 spirochaetes/mL.

Mice inoculation

Two studies were conducted using different strains of mice, different inoculation methods and different times to follow the disease progression as shown in Table 1.

Table 1. Outlining the study design

Experiment no. 1				
Strain	No of animals	Inoculation way	Monitoring period	Euthanized animals
BALB/c	5	intradermally	6 weeks	1 mouse BALB/c+ 1 mouse CD1
			8 weeks	2 mice BALB/c+ 2mice CD1
CD1	5		13 weeks	2 mice BALB/c+ 2mice CD1
Experiment no.2				
BALB/c	12	intradermally	1 week	3 mice ID+3 mice IC
		intracerebral	2 weeks	3 mice ID+3 mice IC

For each experiment, animals were anaesthetized with a mixture of ketamine (65 mg/kg, Vetased, Farmavet, Bucharest, Romania) and xylazine (5 mg/kg, Xylazine Bio 2%, Bioveta, Cluj-Napoca, Romania). The cervical and toraco-dorsal fur was trimmed and disinfected with 3% Iodine. Inoculation of spirochetes was performed in a laminar flow hood. For intradermal inoculation, a volume of 100 μL of borrelia medium, introduced as an intradermal bleb, was used in 10 different points, with orientation as close as possible to the cervical area. Intracerebral inoculation was performed by introducing a 10 μL volume of borrelia, using the same inoculation method described in another study of ours that involved injecting bacteria intra cisterna magna (Coman, 2023). After inoculation, animals were placed in clean cages and maintained in a noise-free environment to recover from anesthesia. At the end of each monitoring period, animals were euthanized by anesthetic overdose. Samples of dura mater, brain, bladder, ear, knee joint, and heart were collected from intradermally inoculated animals, and only dura mater and brain were collected from intracerebrally inoculated animals (Figure 1). Each organ was cut into smaller pieces and deposited in tubes with BSK-H culture medium, then incubated at 33ºC (Figure 2).
The tubes were examined macroscopically daily for signs of contamination.
Microscopic examination under fluorescence microscopy was performed twice weekly over a 6-week incubation period. For each positive tube, the number of incubation days until growth detection, growth quantification and other culture characteristics were noted.

Figure 1. Harvesting of dura mater samples (A) and its fragmentation for deposition in culture medium (B)

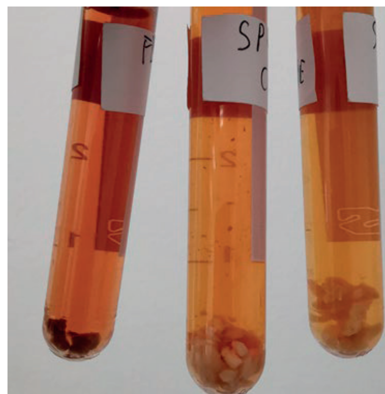
Figure 2. Brain, dura mater and organ samples harvested on BSK-H medium

RESULTS AND DISCUSSIONS

In terms of clinical signs, regardless of the route of inoculation or strain used, we did not observe any obvious symptoms. Two BALB/c mice showed a heavy gait, lameness on a hind limb, 4 weeks after intradermal inoculation, but these symptoms recovered 6 weeks after infection.
Following infection of the mice by intradermal inoculation, in the first experiment, we identified spirochete growth in tubes with medium containing ear samples in BALB/c animals and ear, bladder and knee joint samples in CD1, at the time of sample collection 6 weeks after infection. Growth in the tubes could be observed 20 days after sample collection.
Related to the analysis of samples collected at 8 and 13 weeks, no growth was observed in the tubes, even after 2 months of incubation (maximum follow-up period was 3 months). The number of animals involved in our first experiment is minimal, unlike other studies that used more animals that were followed for longer. Thus, if in the case of our experiment, the *Borrelia* showed an increase in the tube at 20 days after harvesting and no increase at the other times we can hypothesize that the surveillance interval is small compared to Barthold's study when he was able to isolate spirochetes even at 360 days after harvesting (Barthold, 1993) or even that the detection method approached did not detect spirochetes possibly due to the sensitivity limitation and the very small number of spirochetes (Table 2).

Table 2. The results of the first experiment cultural examinations

Samples	6 weeks		8 weeks				13 weeks			
	CD1	BALB/c	CD1	BALB/c	CD1	BALB/c	CD1	BALB/c	CD1	BALB/c
Dura mater	-	-	-	x	-	-	-	-	-	-
Brain	-	-	-	-	-	-	-	-	-	-
Ear	+	+	-	-	-	-	-	-	-	-
Bladder	+	-	-	-	-	-	-	-	-	-
Heart	-	-	-	-	-	-	-	-	-	-
Joint	+	-	-	-	-	-	-	-	-	-
+ (growth of spirochetes); - (no growth of spirochetes); x (contaminated sample)										

Previous studies have shown that the dissemination of B.b. and the severity of disease expression in laboratory mice is dependent on the route of inoculation. Therefore, in the second experiment we wanted to compare the intradermal and intracerebral route of inoculation, the aim being to identify the most efficient method by which borrelia reach the brain and express disease. Thus, the results showed that, one week after inoculation, 2 mice from each intracerebrally or intradermally inoculated group had borrelia in the dura mater samples collected. In animals sacrificed at 2 weeks post-inoculation, 3/3 intracerebrally inoculated animals showed borrelia in the dura mater, the increase being observed after 9 and 15 days of incubation, and in intradermally injected mice, no animal had B.b. positive samples (Table 3).

Table 3. The results of the second experiment cultural examinations

Samples	Intracerebral inoculation					
	1 week			2 weeks		
	Mouse no. 1	Mouse no. 2	Mouse no. 3	Mouse no. 1	Mouse no. 2	Mouse no. 3
Dura mater	+	X	+	+	+	+
Brain	-	-	-	-	-	-
Samples	Intradermally inoculation					
	1 week			2 weeks		
	Mouse no. 1	Mouse no. 2	Mouse no. 3	Mouse no. 1	Mouse no. 2	Mouse no. 3
Dura mater	+	+	-	-	-	-
Brain	-	-	-	-	-	-
Ear	+	-	-	-	x	-
Bladder	-	-	-	-	-	-
Heart	-	-	-	-	-	x
Joint	-	-	-	x	-	-
+ (growth of spirochetes); - (no growth of spirochetes); x (contaminated sample)						

As in an experiment by Divan et al., *Borrelia* were found in the dura mater at both 7 days and 14 days post intracerebral inoculation, in contrast to intradermal inoculation where we isolated spirochetes at 7 days but not at 14 days showing similarity to results reported during late disseminated infection (Divan, 2018).

Spirochete infection can disseminate systemically, reaching many tissues including the CNS where it produces lymphocytic meningitis, cranial neuritis or radiculoneuritis and facial paralysis (Ford, 2021; Eckman, 2018). On our mouse model, signs of nerve damage were not observed although through cultural examinations we were able to identify borrelia in the dura mater. This conclusion may correlate with the situation seen in humans, where approximately 15% of patients infected with B.b. show long-term neurological and psychological symptoms that do not respond to antibiotics (Rauer, 2020).

CNS damage occurs in many infections and often produces irreversible neurological damage (John, 2015). Spirochetes present in the CNS are important indicators for the onset of neuroinflammation (Koedel, 2015). As in our studies we identified borrelia in the brain in the first and second week after inoculation, we can

say that as time goes by, the infection enters a latent phase, so that at 6 weeks post-inoculation we found it in the tissues (experiment number 1), and at 8 and 13 weeks, most likely the spirochete titer decreased below the detection limit by the method of culturing the collected samples.

CONCLUSIONS

In these studies, we were able to develop neuroboreliosis through the inoculation of B.b. on two strains of mice, CD1 and BALB/c, with analysis of tissue samples collected at different time intervals demonstrating the systemic spreading action of *Borrelia*. Also, the intradermal and intracerebral inoculation route of B.b. in the two mouse strains were shown to be able to replicate the disease, which gives us a solid support for testing new drugs against Lyme disease.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CCCDI-UEFISCDI, project number ERANET- EURONANOMED – 3, ANTINEUROPATHO within PNCDI III.

REFERENCES

Baranton, G., Postic, D., Saint Girons, I., et al. (1992). Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. *nov*., and group VS461 associated with Lyme borreliosis. Int J Syst Bacteriol., 42(3):378-383.

Barthold, S.W. (1991). Infectivity of *Borrelia burgdorferi* relative to route of inoculation and genotype in laboratory mice. J lnf Dis, 163:419.

Barthold, S.W., de Souza, M.S., Janotka, J.L., Smith, A.L., & Persing, D.H. (1993). Chronic Lyme borreliosis in the laboratory mouse. The American journal of pathology, 143(3), 959–971.

Barthold, S.W., Sidman, C.L., Smith, A.L. (1992). Lyme borreliosis in genetically resistant and susceptible mice with severe combined immunodeficiency. Am J Trop Med Hyg, 47:605-613.

Casselli, T., Divan, A., Vomhof-DeKrey, E.E., Tourand, Y., Pecoraro, H.L. Brissette, C.A. (2021). A murine model of Lyme disease demonstrates that *Borrelia burgdorferi* colonizes the dura mater and induces inflammation in the central nervous system. PLoS pathogens, 17(2), e1009256.

Coman, C., Ancuța, D.L., Ioniță, F., Muntean, A.A., Caracoti, C.Ș., Caracoti, I.V., Dragomirescu, C., Popa M.I. (2023). Preliminary results in inducing meningitis in BALB/c mice using a human strain of *Neisseria meningitidis*. Scientific Works. Series C. Veterinary Medicine. Vol. LXIX (1), 199-205.

Divan, A., Casselli, T., Narayanan, S.A., Mukherjee, S., Zawieja, D.C., Watt, J.A., Brissette, C.A., & Newell-Rogers, M.K. (2018). *Borrelia burgdorferi* adhere to blood vessels in the dura mater and are associated with increased meningeal T cells during murine disseminated borreliosis. PloS one, 13(5), e0196893.

Eckman, E.A., Pacheco-Quinto, J., Herdt, A.R., & Halperin, J.J. (2018). Neuroimmunomodulators in Neuroborreliosis and Lyme Encephalopathy. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America, 67(1), 80–88.

Ford, L., Tufts, D.M. (2021). Lyme Neuroborreliosis: Mechanisms of B. burgdorferi Infection of the Nervous System. Brain sciences, 11(6), 789.

Garcia-Monco, J.C., Benach, J.L. (2013). A disconnect between the neurospirochetoses in humans and rodent models of disease. PLoS pathogens, 9(4), e1003288.

Grillon, A., Westermann, B., Cantero, P. și colab. (2017). Identificarea proteinei *Borrelia* candidate în pielea șoarecilor pentru diagnosticul potențial al boreliozei Lyme diseminate. Sci Rep., 7(1):16719. Publicat 2017 Dec 1

John, C.C., Carabin, H., Montano, S.M., Bangirana, P., Zunt, J.R., & Peterson, P.K. (2015). Global research priorities for infections that affect the nervous system. Nature, 527(7578), S178–S186.

Kern, A., Schnell, G., Bernard, Q., Bœuf, A., Jaulhac, B., Collin, E., Barthel, C., Ehret-Sabatier, L., & Boulanger, N. (2015). Heterogeneity of *Borrelia burgdorferi* Sensu Stricto Population and Its Involvement in Borrelia Pathogenicity: Study on Murine Model with Specific Emphasis on the Skin Interface. PloS one, 10(7), e0133195.

Koedel, U., Fingerle, V., & Pfister, H. W. (2015). Lyme neuroborreliosis-epidemiology, diagnosis and management. Nature reviews. Neurology, 11(8), 446–456.

Radolf, J. D., & Samuels, D.S. (2021). Lyme Disease and Relapsing Fever Spirochetes: Genomics. Molecular Biology, Host Interactions and Disease Pathogenesis (Norfolk, UK: Caister Academic Press).

Rauer, S., Kastenbauer, S., Hofmann, H., Fingerle, V., Huppertz, H.I., Hunfeld, K.P., Krause, A., Ruf, B., Dersch, R., & Consensus group (2020). Guidelines for diagnosis and treatment in neurology - Lyme neuroborreliosis. German medical science: GMS e-journal, 18, Doc03.

Shapiro, E.D., Gerber, M.A. (2000). Lyme disease. Clin Infect Dis., 31(2):533-542.

Steere, A.C., Malawista, S.E., Snydman, D.R., et al. (1977) Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three connecticut communities. Arthritis Rheum., 20(1):7-17.

van Dam, A.P., Kuiper, H., Vos, K., et al. (1993). Different genospecies of *Borrelia burgdorferi* are associated with distinct clinical manifestations of Lyme borreliosis. *Clin Infect Dis*., 17(4):708-717.

Zawada, S.G., von Fricken, M.E., Weppelmann, T.A., Sikaroodi, M., Gillevet, P.M. (2020). Optimization of tissue sampling for *Borrelia burgdorferi* in white-footed mice (*Peromyscus leucopus*). PLoS One, 15(1): e0226798.

MISCELLANEOUS