

## A Mouse Model of *Borrelia* Meningitis after Intradermal Injection

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Both young and adult C3H/HeN mice developed meningitis within 3 weeks of intradermal inoculation with a newly identified uncultivable *Borrelia* species, an agent of human relapsing fever. Meningoencephalitis with perivascular infiltrates and plexitis developed at ~25 days after inoculation. Infiltrates were composed of B and plasma cells and monocytes. This model recreated the meningitis associated with spirochetal infections through an intradermal route of infection.

Bacterial meningitis is a significant worldwide health problem. Although there are suitable animal models for meningitis with gram-positive and gram-negative bacteria, many of these models rely on direct intracisternal inoculation of the microorganisms [1]. Animal models established by a natural route of infection can provide significant information on pathogenesis, which in turn could lead to a better understanding and management of these infectious disorders of the nervous system.

Tickborne pathogenic *Borrelia* species can be divided into two main types: those that are the etiologic agents of Lyme borreliosis, transmitted by hard ticks (Ixodids), and those that are the etiologic agents of the relapsing fevers, transmitted by soft ticks (Argasids) [2]. The recent discovery of *Borrelia lonestari*, which is phylogenetically similar to agents that cause relapsing fever, in hard ticks suggests that this distinction may not be so clearly defined [3]. The relapsing fever *Borrelia* species occupy a worldwide niche with phylogenetically distinct Old World and New World groupings [2].

We present a model of meningitis that results from the intradermal inoculation of a new species of Old World tickborne relapsing fever *Borrelia* [4]. This murine model might also prove useful for the better understanding of the pathophysiologic mechanisms of neuroborreliosis, as there are significant neurologic similarities between Lyme disease and relapsing fever, notably meningitis and facial palsy [2, 5, 6].

### Materials and Methods

**Bacteria and mice.** The relapsing fever *Borrelia* species used in this study was isolated from the blood of a patient with fever,

arthralgia, and headache in a community in southern Spain in 1994. This *Borrelia* species has a still unclassified taxonomic status but clearly belongs to the Old World group of relapsing fever *Borrelia* species. Phylogenetic studies using the 16S rRNA and flagellin genes indicated that this organism is a new species of pathogenic *Borrelia* [4]. Adult C3H/HeN (3 months old, of both sexes) mice were used primarily for this study [7]. In addition, 3 mice each of both young and adult BALB/c, C57Bl/6, and Swiss outbred mice were used as well. All mice were purchased from Taconic Farms (Germantown, NY).

**Development of infection in the mouse.** The *Borrelia* species was maintained by mouse-to-mouse blood passage of organisms from the first peak of spirochetemia, which occurs on days 5 and 6 after intradermal inoculation of 10<sup>5</sup> spirochetes. This species has been refractory to in vitro cultivation. Occasionally, frozen stocks of the spirochetes were used for inoculations (frozen in liquid nitrogen in mouse plasma with 10% glycerol). An inoculum of 10<sup>5</sup> spirochetes was administered intradermally to the dorsum of shaved mice. The blood of mice was examined daily for spirochetes by darkfield microscopy for 30 consecutive days and expressed as number of organisms per ×40 field. A total of 24 brains was collected from infected mice on days 18, 20, 22, 24, 28, and 30 after inoculation (4/day). The brains were embedded in paraffin and stained with hematoxylin-eosin and by Dieterle's silver stain. Twelve brains from uninfected mice of the same strain, age, and vendor were processed for histology in the same manner as the brain from the infected mice. Frozen sections of brains from mice at various times after inoculation as well as controls were stained with fluorescein- or rhodamine-conjugated goat anti-mouse CD4 (T helper), anti-mouse CD8 (T suppressor), and anti-mouse CD45R or anti-mouse immunoglobulin (B cells) (Boehringer Mannheim, Indianapolis).

### Results and Discussion

Meningitis was a consistent finding in all 24 C3H/HeN adult mice inoculated intradermally with the *Borrelia* species. None of the control mice kept for the same period of time and housed under identical conditions had any evidence of meningitis after histologic examination of the brain. Mononuclear cells infiltrated the leptomeninges (figure 1A) and the choroid plexus (figure 1B). Infiltrates were first noted 18 days after intradermal inoculation. All 24 mice examined had had meningeal and

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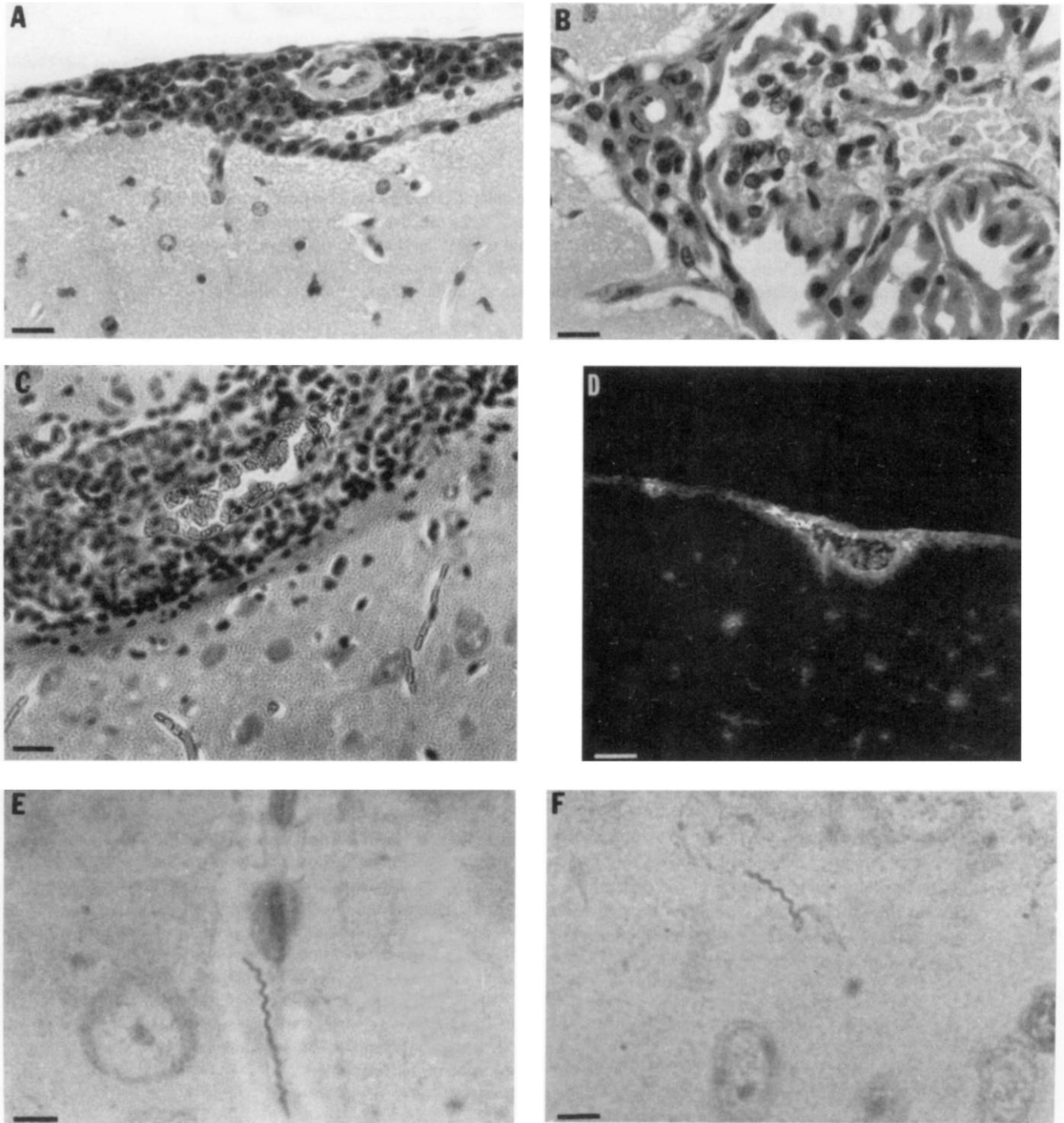
Animal experimentation guidelines for the State University of New York at Stony Brook's Division of Laboratory Animal Resources were followed.

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**Figure 1.** Sections from adult C3H/HeN mice inoculated intradermally with  $10^5$  *Borrelia* species. **A**, Hematoxylin-eosin–stained section of brain from mouse at 21 days after inoculation showing deep mononuclear cell infiltrate in the subarachnoid space. Bar = 14  $\mu$ m. **B**, Plexitis in mouse 22 days after inoculation. Hematoxylin-eosin stain; bar = 12  $\mu$ m. **C**, Hematoxylin-eosin–stained section of brain from mouse inoculated 28 days earlier showing perivascular infiltrate extending into brain tissue. Bar = 14  $\mu$ m. **D**, Meningeal infiltrate stained with fluorescein-labeled anti-mouse CD45R showing predominance of B cells at 22 days after inoculation. Bar = 22  $\mu$ m. **E**, Dieterle’s-stained section of brain showing *Borrelia* species within blood vessel. Bar = 7  $\mu$ m. **F**, Dieterle-stained section of mouse brain showing *Borrelia* species apparently outside brain vasculature. Bar = 7  $\mu$ m.

choroid plexus infiltrates, but variability in histopathologic severity was observed. In some mice, the infiltrates could be seen throughout the leptomeninges, often deep into the sulci, whereas other mice showed only focal areas of perivascular meningeal inflammation. Meningoencephalitis was noted in mice beginning 24 days after inoculation, with mononuclear cell infiltrates in a predominantly perivascular distribution (figure 1C). None of the mice had any obvious signs of physical illness. Although not examined sequentially, both young and adult C3H/HeN, BALB/c, C57Bl/C6, and Swiss outbred mice showed meningeal infiltrates within 20–30 days after inoculation (data not shown).

Plasma cells, B lymphocytes, and monocytes made up the majority of the cell infiltrates, as shown by the reactivity to anti-mouse immunoglobulin and anti-mouse CD45R (figure 1D). CD4 and CD8 cells were rare, as were neutrophils. This composition of the meningeal infiltrates in the mice is similar to the composition of cellular elements in cerebrospinal fluid in syphilis meningitis, a disease caused by another spirochete, *Treponema pallidum* [8].

Silver stains (Dieterle's) of brain sections disclosed numerous intravascular spirochetes (figure 1E). However, determining the presence of spirochetes in the brain itself was more difficult, since the procedures that stain spirochetes (Dieterle's) are notoriously poor for morphologic detail. Figure 1F shows a spirochete that appears to be within the brain tissue.

The experimental infection of mice with this organism has three distinct and decreasing peaks of spirochetemia, with each peak followed by the virtual absence of organisms from the blood [4]. At the inoculum used for this study, the three peaks occur 5–6, 11–13, and 17–22 days after inoculation [4]. Thus, the meningitis in this mouse model begins to develop during or immediately after the third peak of spirochetemia.

While the severity of the meningitis was variable, this condition developed reliably in all mice studied thus far, irrespective of their age or genetic background.

Nonhuman primates show inflammatory responses in the central and peripheral nervous systems after inoculation with *Borrelia burgdorferi*, the agent of Lyme disease [9, 10]. Infection of the brain parenchyma and subarachnoid space of mice has not been documented with *B. burgdorferi* but has been reported with *Borrelia hermsii* (agent of American relapsing fever) by culture and polymerase chain reaction [11]. Thus, this mouse model of spirochetal meningitis will be useful to study the pathophysiology of neuroborreliosis.

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