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Case Report

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Alzheimer Plaques visualized by in situ DNA Hybridization with Molecular Beacons specific for Borrelia – a novel histomorphologic application

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Abstract

Background: *This case describes a novel application of Molecular Beacons, which are a patented technology, for the detection of DNA in tissue sections from an infectious microbe, namely Borrelia burgdorferi, the etiologic agent of Lyme borreliosis. A 65-year-old man with Alzheimer's disease and previously well documented spinal fluid neuroborreliosis eight years prior to death is the subject of this report. Neuroborreliosis in its tertiary from has been linked to some cases of Alzheimer's disease (1. -4.)*

Findings: *Molecular beacons designed from the flagellin b open reading frame (BBO 147) of Borrelia burgdorferi, strain B31 demonstrated positive fluorescein signals indicating successful probe hybridization with discrete 4 sharply demarcated rounded foci within tissue slides from autopsy hippocampus.*

Conclusions: *Molecular beacons, carefully designed to hybridize only with the DNA of a target pathogen (after a comprehensive search of the entire human genome to confer probe specificity) are powerful molecular interrogators for evidence of tissue infection. The implications of the application are far reaching in the study of neurodegenerative diseases which might (like General Paresis and Tabes Dorsalis) be sequelae of chronic bacterial infection in the central nervous system.*

Abbreviations: DNA, NCBI, PBS, SUNY, CERAD, NCBI

Findings: The patient reported here was initially found at the age of 59 years to have Borrelia burgdorferi infection in his spinal fluid. This diagnosis was made at the School of Medicine, State University of New York at Stony Brook, Stony Brook, New York. He did not demonstrate cutaneous, cardiac, or rheumatologic findings of Lyme Borreliosis. In 1997 he received intravenous Ceftriaxone (Rocephin) for three weeks. The patient's neurologic function deteriorated. Spinal fluid studies (Enzyme linked immunosorbent assay and Western Blot) continued to demonstrate high titer antibodies to Borrelia burgdorferi. In the ensuing eight years his deterioration was marked by the development of Hydrocephalus, treated with a ventriculoperitoneal shunt. Shunt infection developed and additional parenteral antibiotic therapy was administered. Neuropsychiatric and cognitive function decline, personality changes, visual, and auditory hallucinations ensued. He died in 2005.

Autopsy study: An autopsy was requested by the family. On the autopsy consent form the patient's spouse specifically indicated "history of Lyme disease". Neuropathologic examination was completed by a Pathologist with subspecialty certification in Neuropathology from the American Board of Pathology and a faculty member of the Medical School of the SUNY Stony Brook. The final autopsy findings ratified Alzheimer's disease based on CERAD criteria. Immunostains for Tau protein and Beta Amyloid were completed. No attempt to apply immunohistochemical staining for Borrelia burgdorferi was undertaken by the faculty of the School of Medicine, State University of New York at Stony Brook, according to the final autopsy report. Molecular Beacon studies: At the request of the spouse (CD) unstained paraffin sections from the hippocampus region CA1 were submitted to one of us (ABM) for an attempt to detect residual Borrelia burgdorferi. A novel approach for the detection of the DNA of the Borrelia burgdorferi spirochete, namely Molecular Beacons.

Designed and manufactured to detect a portion of the flagellar b open reading frame BBO147 were utilized for molecular interrogation of autopsy tissues. A 22 nucleotide sequence was designed by Alan B. MacDonald,M.D. and the "stem" sequences connecting Fluorescein and the quencher molecule Dabcyl were designed by Ali Javed Ph.D. and manufactured by Gene Link Inc., Hawthorne, N.Y. (www.genelink.com). Software analysis for the DNA probe sequence and molecular beacon design were facilitated by Primer Premiere© (www.premierebiosoft.com)

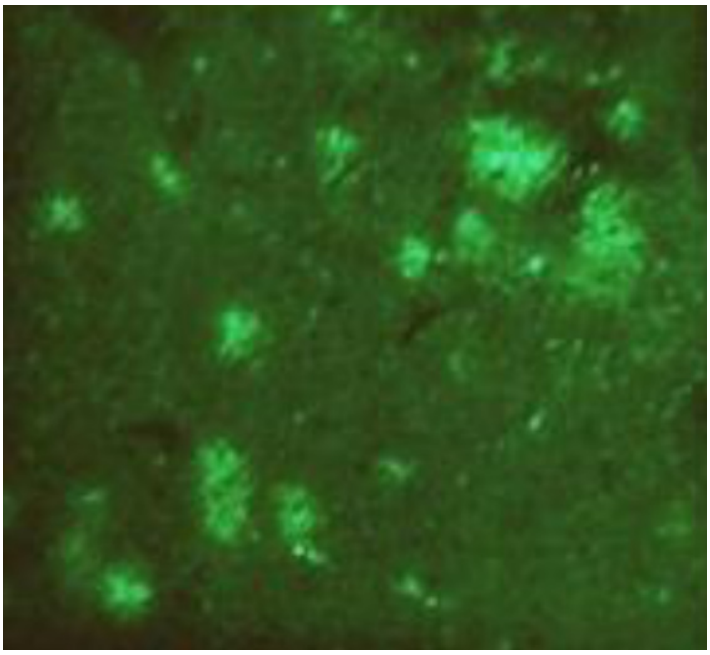

Figure 1: Alzheimer plaques in autopsy Brain bind DNA probe for Fla B Gene (FISH method) TGG GAG TTT CTG GTA AGA TTAA

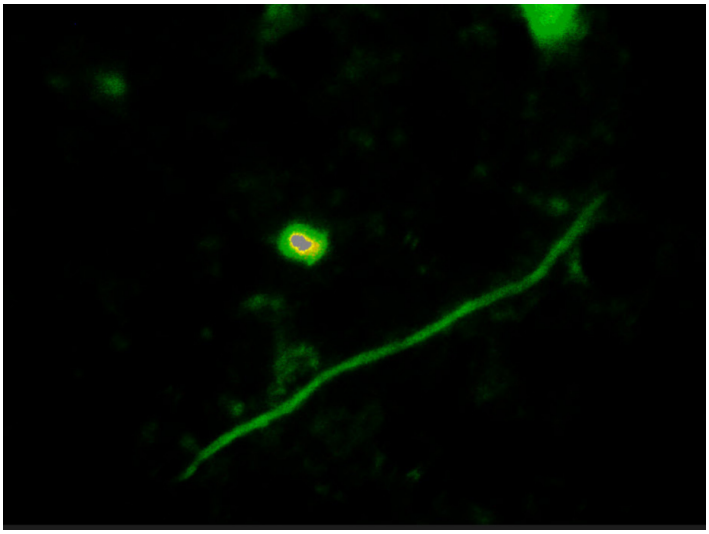

Figure 2: Borrelia spirochete in Alzheimer's Brain binds Flagellin B DNA probe (FISH method) TGG GAG TTT CTG GTA AGA TTAA

DNA Hybridization Protocol: The slides were deparafinized by baking in an oven at 60 degrees followed by passage through serial dwellings in 100% xylene, and graded alcohol solutions (100%,100%,95%,80%) and finally transferred to distilled water in the usual manner for preparation of sections embedded in wax and cut at three-micron section thickness for histologic study. The decerated tissues were allowed to air dry at 24 degrees centigrade. The tissue sections were flooded with the Molecular Beacon in TE buffer as received from the manufacturer (Gene Link Inc., Hawthorne, New York).

Glass coverslips were then carefully placed over the tissue beacon flooded area. The slides were then placed on the heating block of a Perkin Elmer Thermocycler and heated to 90 degrees centigrade to denature all DNA. The slides were then removed from the heating block and allowed to return to room temperature. The coverslips were then removed and the slides were washed in three changes of sterile phosphate buffered 0.9% saline pH 7.0. (Cambrex Biologicals Inc.) A solution of 30% glycerol in phosphate buffered saline was applied and glass coverslips were placed over the tissue in the usual manner. The glass coverslips were carefully compressed between two paper towels to remove all air bubbles and excess glycerol solution.

Positive Controls for the Molecular Beacon: Air dried fixed smears of a pure culture of Borrelia burgdorferi were the positive controls for this study (received from Dr. Tom Schwan, Rocky Mountain Laboratory, National Institutes of Allergy and Infectious Disease, Hamilton, Montana. These control slides were reacted with the identical molecular beacons in a manner identical to the tissue slides. Epifluorescent microscopy with a Nikon Labophot microscope outfitted with an appropriate excitation / barrier filter cube was utilized at 500x and 1000x oil immersion final magnification was used to examine the patient's slides and the positive control slides. Photographs were obtained through the microscope's trinocular head and camera apparatus.

Results

Discrete sharply demarcated rounded areas of fluorescein fluorescence were detected in the patient's tissue sections after hybridization with the Molecular Beacon. The size, contour, geographic placement of Molecular Beacon positive areas in the patient tissues was compatible with the Alzheimer argyrophilic plaque distribution in the conventional autopsy sections. The positive control slides demonstrated fluorescein fluorescence with a clean background in Borrelia burgdorferi spirochetes (spiral forms) [1-3].

Specificity of the Molecular Beacons

The Flagellin b nucleotide sequence (22 bases) was shown to be specific for Borrelia by a BLAST supercomputer search of the entire human genome, which is on deposit at the National Center for Biotechnology (NCBI) (www.ncbi\BLAST). No 100% identical 22 nucleotide sequence exists in the normal human genome database in the NCBI. Molecular Beacons are patented probes with such high specificity that, by definition, the Beacons will only emit fluorescence when a 100% nucleotide match is present in a specimen with diverse DNA sequences present. By definition in the patent for Molecular Beacons, a mere mismatch of a single nucleotide will prevent the beacon from undergoing the conformational changes which move the "arms" with the attached fluorochrome molecule away from its adjacent quencher molecule (in this case Dabcyl) (i.e. a single nucleotide polymorphism (SNP) is sufficient to prevent a beacon from hybridizing with an intended target nucleotide sequence) [4, 5].

Conclusion

This case represents the first report of a patient with well documented spinal fluid neuroborreliosis treated and followed for eight years at a major academic medical center with an international recognition as a Lyme disease center of excellence, which terminated in death due to Alzheimer's disease (confirmed at autopsy study in the School of Medicine). This is also the first case of the utilization of Molecular Beacons as histopathologic stains specific for the detection of the flagellin b DNA of Borrelia Burgdorferi in diseased human brain tissue.

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