

#### Immunohistochemistry in the diagnosis of Lyme Disease

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by Bahram Robert Oliai, M.D.

In this month's FOCUS we examine an exciting new application of immunohistochemistry to infectious disease.

Borreliosis (Lyme Disease) is a systemic illness resulting from infection with the spirochete *Borrelia burgdorferi* which is transmitted by ticks (*Ixodes scapularis* and *Ixodes pacificus* in the U.S. and *Ixodes ricinus* in Europe). The disease is endemic to large portions of the United States with 23,305 cases reported in 2005 (CDC data). There are three clinical stages of Lyme Disease and skin lesions are an essential feature in most cases. Stage 1 is characterized by erythema migrans (EM), the classic expansile targetoid skin rash of Lyme disease (Fig. 1). In addition, Lymphocytoma cutis, a florid dermal B cell reaction, often with germinal center formation can be seen in early stage Lyme Disease, although this seems more common in European cases for some reason. Other skin lesions manifesting in early stage Lyme Disease can show histological patterns similar to granuloma annulare, morphea, papular urticaria, and Henoch Schoenlein purpura. Stage 2 is dominated by arthritis, meningoradiculitis, and carditis. Finally, the hallmark of Stage 3 Lyme Disease is acrodermatitis chronica atrophicans (ACA). Although these are the classic presentations, Lyme Disease can have protean manifestations also including vague symptoms related to musculoskeletal and internal organ involvement.

The diagnosis of Lyme Disease is generally based on classic symptoms, objective examination findings (such as EM, arthritis, Bell's palsy) and exposure to a tick. Unfortunately, clinical diagnosis can be problematic as patients may not develop classic EM, may

manifest unusual or vague symptoms, and may not recall or even realize a possible tick bite (especially since EM can occur weeks afterwards). In addition, even in cases in which a targetoid rash is present the differential diagnosis can include various gyrate erythemas such as erythema gyratum repens and erythema annulare centrifugum. Given these issues, laboratory testing can play a prominent supportive role in making the diagnosis.

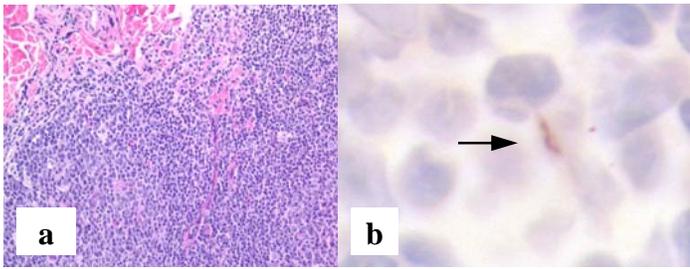
Serologic tests (i.e. ELISA or IFA) are popular. However because the host antibody response to *B. burgdorferi* develops slowly, only half of patients with early-stage Lyme disease will have a positive serology. False negative rates of 20-80% have been reported. In addition, these serologic test can show cross reaction with another infamous spirochete, Syphilis, leading to false positive results. Much like Syphilis, a positive Lyme IgG remains, even after successful treatment, and therefore results must always be interpreted in the context of the patient's history.

*Borrelia* can be cultured, however they are fastidious requiring special media and cultures have shown a sensitivity of only 50%.



**FIG. 1**

The classic targetoid skin rash of erythema migrans (from Google).



**FIGS. 2a&b** Lyme organisms (b, arrow) as detected by immunohistochemistry in a Borrelial lymphocytoma (a).

Because Lyme Disease has many dermatologic manifestations and laboratory testing can be unsatisfying, a method of directly identifying organisms in paraffin embedded tissue sections would be of great potential use. In the past a number of special stains have been utilized for this purpose (Warthin Starry A.K.A “Worthless Starry”, Wright Giemsa, etc.) I can attest to the fact that these are very difficult stains to interpret often due to nonspecific and high background staining. This is why I have long thought that immunohistochemistry could be a much more effective tool in identifying these spirochetes, which are often few in number, and hidden in a florid inflammatory reaction. Unfortunately, there is little data available concerning immunohistochemistry in the detection of *Borrelia* and until recently, I knew of no good source for positive control material for Lyme. In fact, I have even tried injecting live culture strains of Lyme into pieces of surgical tissue and fixing them attempts to produce a good positive control.

This month we introduce our new and effective anti-*borrelia* antibody, and discuss the potential application of immunohistochemistry in the diagnosis of Lyme Disease.

In the February 2007 issue of the *American Journal of Clinical Pathology*, Drs. Eisendle, Grabner, and Zelger of the Innsbruck Medical University in Austria, described the successful detection of *Borrelia* from both paraffin embedded ticks and various skin lesions (including tick bites, EM, borrelial lymphocytomas, and ACA). Specifically, they used a polyclonal rabbit antibody and a technique called focus

floating microscopy (in which the microscope is focused up and down through the section at high power). Results of immunohistochemistry (sensitivity 96%, specificity 99.4%) compared quite favorably to PCR (sensitivity 45.5%, specificity 100%) from the same tissues.

Recently, thanks to the generosity of Drs. Eisendle, Grabner, and Zelger in providing us with appropriate control material from their innovative study and their clinic in Innsbruck, we have been able to finally successfully titer our own Lyme Disease antibody (Fig. 2). As Lyme is a spirochete, our antibody will cross react with Syphilis, which should be kept in mind, especially if Syphilis is in the differential diagnosis and has not been otherwise ruled out. Additionally, in working up the antibody we noticed that it did show staining of several cocco-bacillary bacteria in our own bacteria/fungi control block, however these were from a case of pneumonia, and did not have the curved or spiral morphology of a spirochete. Finally, as with virtually any immunostain for an infectious organism, a negative result does not necessarily rule out infection and results must always be interpreted in the setting of the clinical findings.

We are now offering the Lyme Disease antibody clinically and are excited to provide this potentially useful immunostain to our clients with the service and turn around time for which ProPath is famous.

#### **Acknowledgements:**

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*Bahram Robert Oliai M.D.*

Dr. Bahram Robert Oliai is the newest addition to ProPath’s IHC Laboratory and Consultation Services. After graduating from the University of Illinois College of Medicine, Dr. Oliai completed an Anatomic and Clinical Pathology residency and Surgical Pathology fellowship at The Johns Hopkins Hospital. He then completed a second fellowship at the Memorial Sloan Kettering Cancer Center in Cytopathology. Before joining ProPath, Dr. Oliai was in private practice in Chicago for several years and did additional subspecialty training in Urologic Pathology at The Johns Hopkins Hospital. Dr. Oliai is board certified in Anatomic and Clinical Pathology and Cytopathology. For direct inquiries to Dr. Oliai, contact him at [Robert.Oliai@propathlab.com](mailto:Robert.Oliai@propathlab.com)

**PROPATH**

The Leader in Pathology Services

8267 Elmbrook Dr, Ste 100  
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(800) 258-1253

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