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Research Paper

Borrelia burgdorferi-specific IgA in Lyme Disease

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ABSTRACT

The laboratory diagnosis of Lyme disease is currently dependent on the detection of IgM and IgG antibodies against *Borrelia burgdorferi*, the causative agent of the disease. The significance of serum IgA against *B. burgdorferi* remains unclear. The production of intrathecal IgA has been noted in patients with the late Lyme disease manifestation, neuroborreliosis, but production of antigen-specific IgA during early disease has not been evaluated. In the current study, we assessed serum IgA binding to the *B. burgdorferi* peptide antigens, C6, the target of the FDA-cleared C6 EIA, and FlaB(211-223)-modVlsE(275-291), a peptide containing a Borrelia flagellin epitope linked to a modified VlsE sequence, in patients with early and late Lyme disease. Specific IgA was detected in 59 of 152 serum samples (38.8%) from early Lyme disease patients. Approximately 50% of early Lyme disease patients who were seropositive for peptide-specific IgM and/or IgG were also seropositive for peptide-specific IgA. In a subpopulation of patients, high peptide-specific IgA could be correlated with disseminated disease, defined as multiple erythema migrans lesions, and neurological disease complications. These results suggest that there may be an association between elevated levels of antigen-specific IgA and particular disease manifestations in some patients with early Lyme disease.

1. Introduction

Lyme disease (LD) is a tick-transmitted bacterial infection caused by spirochetes of the genus Borrelia, including *B. burgdorferi* (Bb), *B. garinii*, and *B. afzelii*. It is endemic in parts of North America and Europe. The skin lesion, *erythema migrans* (EM), is a classic marker of early infection and present in ~80% of acutely diagnosed individuals (Steere et al., 1998). It is the only specific clinical marker for LD (Wormser et al., 2006; Steere et al., 1998) and in regions endemic for LD, presentation with EM is considered diagnostic. Other clinical manifestations are non-specific and are found in a wide variety of other illnesses. Unlike most bacterial infections where culture is the major diagnostic method, culture of Bb has proven to be ineffective for routine use (Centers for Disease Control and Prevention (CDC), 1995). Therefore, the laboratory diagnosis of LD is based on indirect methods, primarily the serological detection of IgM and IgG antibodies against Bb (Schriefer, 2015). In North America, seroreactivity is tested using the two-tier paradigm delineated by the CDC, consisting of a first-tier EIA and a second-tier immunoblot measuring IgM and IgG (Centers for Disease Control and Prevention (CDC), 1995; Craven et al., 1996).

The two-tier paradigm has excellent specificity; however, low sensitivity is a significant issue in early disease. The sensitivity of current IgM and IgG LD assays during early disease seldom exceeds 50% (Stanek et al., 2012; Nowakowski et al., 2001; Gomes-Solecki et al., 2001, 2002; Liang et al., 2004; Wormser et al., 1999; Bacon et al., 2003; Coulter et al., 2005; Robertson et al., 2000). For those patients that either do not develop an EM, or present with an atypical EM that can be mistaken for a rash, there is a significant need for sensitive and accurate laboratory diagnostics for Borrelia infection (Schutzer et al., 1999). Early intervention is paramount for ensuring good patient outcomes and preventing development of subsequent late stage disease that can result in permanent damage to neurological and musculoskeletal systems (Aguero-Rosenfeld et al., 2005). That Bb induces the generation of specific IgM and IgG antibodies is well documented. However, the role of serum anti-Bb IgA in early LD patients has not been defined.

IgA is the second most common antibody isotype in human blood, after IgG. Unlike polymeric IgA produced at mucosal surfaces, human serum IgA is principally monomeric (subclass IgA1). Monomeric serum IgA is not secreted across the mucosal barrier and has a half-life of 4.5–6 days in peripheral blood (Schaller et al., 2008). The role of serum IgA in immunity has yet to be clearly defined. With respect to LD, one publication that focused on the development of Lyme arthritis and cryoglobulin IgM in 48 untreated EM patients had incidentally noted that circulating anti-Borrelia IgA and IgG tended to move conversely to IgM (Steere et al., 1979). Other studies associating IgA and LD have concentrated on the intrathecal production of anti-Bb IgA in patients with neuroborreliosis (Steere et al., 1990; Kaiser, 1998; Jesse et al., 2011; Schwenkenbecher et al., 2017; Kowarik et al., 2012; Roberg et al.,

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1995; Kaiser, 1998; Jesse et al., 2011; Schwenkenbecher et al., 2017; Kowarik et al., 2012). No study has specifically addressed the presence of circulating anti-Borrelia IgA in early LD. In the present study, we found that approximately one-third of patients presenting with early LD had circulating IgA antibodies specific for Borrelia peptide antigens. We utilized peptides that were highly specific to Bb to limit the detection of cross-reactive IgA induced by a response to a non-Borrelia infection. Our results suggest that there could be a potential role for serum IgA detection in the laboratory diagnosis of LD.

2. Materials and Methods

2.1. Serum Samples

We performed a retrospective analysis of Bb-specific IgA in serum from patients presenting with early LD ($n = 152$) and in patients with Lyme arthritis (late LD) ($n = 19$). Sera from patients with early LD were collected as a convenience series; that is, patients were recruited from populations presenting to seasonal LD clinics (Table 1) typically with one or more EM lesions and receiving a clinical diagnosis of early LD. For patients included in this study that did not present with an EM, diagnosis was supported by clinical presentation and positive standard serology (Table S1 and S2). All collection sites (Table 1) are in regions endemic for LD, and sera were obtained during LD season, which runs from mid-spring to mid-fall. Sera from Lyme arthritis patients were collected from patients upon first clinical presentation to the Gundersen Lutheran Medical Center in LaCrosse, WI; diagnosis was made based on clinical presentation, all had joint swelling and a positive Lyme serology. All sera were collected at the time of initial diagnosis with consent and under institutional review board (IRB) approval from the relevant institutions listed in Table 1. All of the samples were de-identified before being provided to us; experiments were conducted without prior knowledge of clinical symptoms beyond a diagnosis of early Lyme disease. For a limited number of samples, presented in Tables S1 and S2, alphanumeric coding allowed access to de-identified clinical data associated with the sample.

To define the cutoff (CO) values for equivocal and positive antibody levels we assessed antibody binding in sera from healthy individuals collected in both LD endemic and nonendemic areas. We defined positive levels of antibody as a mean absorbance of three replicate wells >3 SD from the mean of the nonendemic healthy controls; equivocal levels were defined as greater than 2 SD but less than 3SD from the mean, and negative levels were defined as <2 SD from the mean. The CO values for IgA positive detection were calculated to be $\text{Absorbance}_{(450-570)}$ 1.769 (0.749 + 3 * 0.340) and $\text{Absorbance}_{(450-570)}$ 0.920 (0.423 + 3 * 0.166) for C6 and FlaB-mV, respectively. The CO values for IgA equivocal detection were calculated to be $\text{Absorbance}_{(450-570)}$ 1.428 (0.749 + 2 * 0.340) and $\text{Absorbance}_{(450-570)}$ 0.754 (0.423 + 3 * 0.166) for C6 and FlaB-mV, respectively. Endemic healthy donors were collected from Long Island, NY, an area where seroconversion was recorded at a rate of ~12% per year (Ginsberg, 2005). These sera were utilized as a separate negative healthy control. Sera from patients with rheumatoid arthritis (RA), fibromyalgia, or syphilis were used as negative disease controls for specificity in this assay. RA sera are used as negative disease controls because joint destruction and large joint inflammation are also observed in LD, while syphilis sera serve as controls for an infectious disease caused by a related spirochete, *Treponema pallidum*. Fibromyalgia sera are used as controls because the disorder is marked by diffuse nonspecific symptoms that are sometimes misinterpreted as early LD. All sera were aliquoted and stored at −80 °C. For the study, individual aliquots were moved to −20 °C, and freeze-thaw cycles were kept to a minimum.

2.2. Peptide ELISA

Peptide antigens, C6 (CMKKDDQIAAAIALRGMAKDGKFAVK) and FlaB(211-223)-modVlsE(275-291) (FlaB-mV) (CVQEGVQQEGAQQ PGGGMKKNDQIVAAIALRGVA) were synthesized by LifeTein (Somerset, NJ). C6 is derived from the Bb membrane protein VlsE, and is the antigen target in the FDA-cleared C6 first-tier EIA. For these studies we utilized a synthesized C6 peptide (Gomes-Solecki et al., 2007) and not the commercially available C6 assay. FlaB-mV is a previously described peptide (Lahey et al., 2015) from the central portion of the Bb flagellin protein linked to a modified VlsE sequence. This sequence is altered to represent a consensus sequence found in VlsE among different Borrelia strains to minimize the impact of sequence variability on antibody binding. ELISAs for IgA, IgM, and IgG binding to peptides were performed as previously described (Arnaboldi et al., 2013) using 10 μg/ml of peptide and (HRP)-labeled goat anti-human IgA, IgM, and IgG (Southern Biotech, Birmingham, AL) as detecting antibodies at 1:15,000, 1:8000, and 1:5000 dilutions, respectively.

Table 1
Patient serum samples.

Patient health status	Obtained from	Number	Region obtained	Lyme disease prevalence
Early Lyme disease[a]	New York Medical College[b]	94	Northeast (New York)	Endemic
	Stony Brook University[c]	20	Northeast (New York)	Endemic
	Gundersen-Lutheran Medical Center[d]	38	Upper Midwest (Wisconsin)	Endemic
Late Lyme Disease[e] (Lyme arthritis)	Gundersen-Lutheran Medical Center[d]	19	Upper Midwest (Wisconsin)	Endemic
Healthy individuals	Creative Testing Solutions[f,g]	64	New Mexico	Nonendemic
	Bioreclamation, LLC[g,h]	40	Southern California	Nonendemic
	Stony Brook University[c,i]	35	Northeast (New York)	Endemic
Rheumatoid arthritis[j]	Bioreclamation, LLC[g]	53	Northeast	Endemic
Syphilis[k]	Bioreclamation, LLC[g]	34	Northeast	Endemic
Fibromyalgia	Bioreclamation, LLC[g]	16	Northeast	Endemic

[a] Sera were collected from patients at their initial presentation to Lyme disease clinics.
[b] Located in Westchester, NY.
[c] Located in Long Island, NY.
[d] Located in LaCrosse, WI.
[e] Sera were collected from patients upon first clinical presentation with swollen joints.
[f] Tempe, AZ.
[g] Sera were commercially purchased.
[h] Westbury, NY.
[i] Collected from healthy individuals working at the Fire Island National Seashore as part of a Lyme disease surveillance study in Long Island, NY (through Stony Brook University).
[j] Rheumatiod factor status unknown.
[k] Syphilis patients had a positive Rapid Plasma Reagin test and anti-treponemal antibody.