IgA Antibody Responses to Borrelia Burgdorferi and Three Specific Autoantigens in Patients With Lyme Disease: Correlations With Cytokine Mediators and Disease Duration

IgA Antibody Responses to *Borrelia Burgdorferi* and Three Specific Autoantigens in Patients with Lyme Disease: Correlations with Cytokine Mediators and Disease Duration

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Abstract

The aim of this research was to investigate IgA antibody responses to *Borrelia burgdorferi* and 3 related autoantigens in patients with early manifestations of Lyme disease, including those with erythema migrans (EM) and in those with antibiotic-responsive and antibiotic-refractory Lyme arthritis (LA), a late disease manifestation. IgA antibody responses were then correlated with levels of innate, Th1 and Th17 inflammatory mediators and with arthritis duration. As determined by ELISA, serum levels of IgA antibodies to *B. burgdorferi* were highest in patients with LA, and these responses were still higher in joint fluid in those with antibiotic-responsive arthritis, suggesting local production of these antibodies in synovial tissue. In contract, IgA autoantibody responses to the 3 autoantigens, endothelial cell growth factor (ECGF) and matrix metalloproteinase-10 (MMP-10), and apolipoprotein B-100 (apoB-100) were similar or greater in serum than in joint fluid, both in response and refractory groups, suggestive of production at extra-articular sites. Serum IgA antibody responses to *B. burgdorferi* in patients with EM correlated directly with the levels of Th17-associated cytokines, whereas IgA responses to the spirochete in joint fluid in patients with refractory LA correlated inversely with the Th17-associated cytokine levels. Furthermore, IgA antibody responses to the 3 autoantigens correlated directory with the duration of arthritis in patients with refractory LA. Thus, in patients with EM or LA, IgA antibody responses to *B. burgdorferi* would appear to play a role in fighting the infection, but IgA responses to the 3 autoantigens appear to be a marker for prolonged arthritis that persists after spirochetal killing with antibiotic therapy.

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Chapter I

Introduction

Background of Lyme disease

Lyme disease is caused by the tick-borne spirochete *Borrelia burgdorferi* and is the most common vector-borne illness in the United States (Steere, 2001). It is the most prevalent in the Northeast, upper Midwest and Western regions of the country. In addition, it is also quite established in the forested areas of countries in Europe, Russia, China and Japan. In the United States, the infection is strictly caused by infection with *B. burgdorferi* while in Europe and Russia, it is as a result of *B*. *afzelii* and *B*. *garinii* infection (Steere, 2001).

Lyme borreliosis in all locations is transmitted by ticks of the *Ixodes ricinus* complex. These ticks have larval, nymphal and adult stages that require a blood meal at each stage (Steere, 2001). The structure of the *Borrelia* species is a protoplasmic cylinder that is surrounded by a periplasm that contains flagella which is then enclosed within an outer membrane. A feature that is unique to the *Borrelia* species is that a number of their outer-membrane proteins are encoded by plasmid genes. The known virulence factors of *B. burgdorferi* are certain surface proteins that allow the spirochete to attach to mammalian cells (Steere, 2001).

Clinical Manifestations and Pathogenesis

During the fall, winter and early spring, the spirochete is in a dormant state in the midgut of a tick. The nymphal stage of the tick, which is primarily responsible for disease

transmission, feeds in the late spring and early summer, particularly in June and July (Steere, 2016). The outer surface protein C (ospC) is upregulated while the tick feeds, OspC expression is necessary in order for the spirochete to infect the mammalian host. The spirochete binds mammalian plasminogen and its activators that are present in the blood meal in order to spread within the host (Steere, 2001). In order for the transmission of the spirochete to occur, 24-72 hours of tick attachment is necessary. Therefore, removing the tick within 24 hours is very effective in preventing Lyme disease. However, if the tick is not recognized and transmission of the spirochete occurs, Lyme disease then occurs in three distinct stages if it is left untreated.

Stage I: Early Infection

Once a person has been infected with the spirochete, the incubation period ranges from 3 to 32 days before a slowly expanding skin lesion begins. This skin lesion, erythema migrans (EM), forms at the site of the tick bite and is generally visible in 70-80% of those who have been infected. The symptoms of this stage of Lyme disease vary in different regions of the world. In the United States, the skin lesion is often accompanied by flu-like symptoms (malaise, headache, fatigue, arthralgia, myalgia and fever) (Steere, 2001). In Europe, EM is more often just a localized infection that does not lead to any other systemic symptoms. When the skin lesion is accompanied by the flu-like symptoms, it suggests that the spirochete has disseminated (Steere, 2001). Interestingly enough, *B. burgdorferi* in the U.S. disseminates more commonly than *B. afelii* and *B. garinii,* which are the primary causes of disease in Europe (Jones et al., 2009).

When *B. burgdorferi* is first present at the site of the tick bite, immune cells identify it. Histological examination of erythema migrans shows that various cells are present at the site, including lymphocytes, dendritic cells, macrophages, and a minimal number of plasma cells (Petzke and Schwartz, 2015). Macrophages engulf and kill the spirochetes as a part of the innate immune response. The inflammatory cells in the lesion produce pro-inflammatory cytokines such as TNF-α and IFN-γ. PBMCs (peripheral blood mononuclear cells) that have been stimulated by *B. burgdorferi* in patients with erythema migrans produce Th1 pro-inflammatory cytokines (especially IFN-γ) (Glickstein, 2003). In the days following the onset of the disease, most patients have an IgM antibody response to the outer surface protein C or the 41-kDa flagellar protein of the spirochete (Steere, 2004). At this stage of the infection, both the innate and adaptive parts of the immune system are mobilized to fight the infection (Steere, 2004).

Stage II: Disseminated Infection

Days to weeks after the onset of the infection, *B. burgdorferi* often disseminates widely throughout the body and the spirochete can be present in blood and cerebrospinal fluid in addition to the myocardium, retina, muscle, bone, spleen, liver, meninges, and brain (Steere, 2001). This results in many other possible clinical manifestations such as secondary annular skin lesions, meningitis, neuropathy, radiculoneuritis, atrioventricular nodal block and musculoskeletal pain the joints, tendon, muscle and bone (Steere, 2001). *B. burgdorferi* disseminates at this point by binding to certain host proteins and adhering to integrins, proteoglycans, or glycoproteins on the host cells or tissue matrices. A 47-kDa spirochetal protein binds fibronectin, an extracellular membrane protein that binds

components such as collagen, fibrin and heparan sulfate proteoglycans (Probert, 1998). Although multiple sequences of outer surface protein C are present among strains, only a few of them are associated with disseminated infection. A 66-kDa outer surface protein of the spirochete binds to fibrinogen receptors and vitronectin receptors in the host, which help the organism establish an initial foothold and disseminate in vasculature (Steere and Coburn, 1999). The *B. burgdorferi* glycosaminoglycan-binding protein, which is 26-kDa, binds to GAG side chains of heparan sulfate and dermatan sulfate on neuronal cells while spirochetal decorin-binding proteins A&B (DbpA & DbpB) bind decorin (Parveen, 2000). Decorin is a proteoglycan that associates with collagen and results in the alignment of spirochetes with collagen fibrils in the extracellular membrane of the heart, nervous system and joints (Guo, 1998).

Despite an active immune system response, *B. burgdorferi* can escape and survive dissemination by changing or minimizing the antigenic expression of its surface proteins (Zhang, 1998). Another way for *B. burgdorferi* to survive is by inhibiting certain critical host immune responses, and immune pressure from the host may cause *B. burgdorferi* to down regulate lipoproteins. For the disseminated infection to be successfully controlled, both innate and adaptive immune responses must both be active (Petzke and Schwartz, 2015). The lipoproteins contained by *B. burgdorferi* are B-cell mitogens and initially stimulate adaptive T cell-independent B cell responses. Nonlipidated spirochetal proteins are more likely to be T cell dependent and the humoral immune response against them is helpful in killing of the spirochetes (Petzke and Schwartz, 2015).

The T cell-dependent B cell responses are optimized by the Th1 cells that are specific to *B. burgdorferi*. The combination of these responses leads to the production of

antibodies against many components of the organism, which promotes spirochetal killing by complement fixation and opsonization (targeting of bacterial cells by phagocytes). This stage lasts anywhere from several weeks to months. The antibody responses plus the innate immune response mechanisms control the widely disseminated infection even without antibiotic treatment and eventually, the generalized symptoms resolve (Steere, 2004).

Stage III: Persistent Infection

After the infection has disseminated, the Lyme disease agent may survive in localized niches for several years (Steere, 2001). At the third stage, systemic symptoms are minimal or even non-existent but it is possible that the infection has previously spread to the joints, nervous system and other skin sites. Months after the onset of Lyme disease, 60% of patients in the United States who were not treated with antibiotics experience intermittent attacks of arthritis, especially at the larger joints such as knees (Arvikar and Steere, 2015). $CD4^+$Th1 cells are proinflammatory and *B. burgdorferi* specific $CD8^+$T cells are also present in human infection. Within the joint, *B. burgdorferi* specific T regulatory cells can help in regulation of the inflammatory responses (Petzke and Schwartz, 2015). People that contract Lyme arthritis have very high antibody responses to many of the spirochetal proteins. This suggests hyper-immunization due to the recurrent waves of spirochetal growth (Steere, 2001). These immune mechanisms seem to be able to eventually eradicate *B. burgdorferi*, and patients who continue to have attacks of arthritis decreases by about 10-20% each year (Steere, 1987). A rare and late neurologic syndrome, Lyme encephalopathy or polyneuropathy, occurs rarely in the US

at this stage. It is manifested primarily by subtle cognitive disturbances, spinal radicular pain or distal paresthesias (Logigian, 1990). However, the duration of spirochetal persistence and pathogenic mechanisms are not clear.

Lyme Arthritis

In late stage *B. burgdorferi* infection, Lyme arthritis is a common occurrence in those who have not been given antibiotic therapy. Patients have attacks of swelling and pain, particularly affecting the knee, which is the most frequently affected joint (Arvikar and Steere, 2015). The arthritis is successfully treated in most patients with appropriate oral or IV antibiotic therapy. However, in a small percentage of patients, joint inflammation persists even after antibiotic therapy for 2-3 months. This is called antibiotic-refractory Lyme arthritis. Patients who are diagnosed with antibiotic-refractory arthritis generally have a combination of pathogen and host-associated genetic and immunologic factors (Arvikar and Steere, 2015). After antibiotic therapy, treatment for patients with antibiotic-refractory arthritis involves treatment similar to those with other forms of chronic inflammatory arthritis, such as rheumatoid arthritis.

Diagnosis & Clinical Testing

For early disease, serologic tests are highly dependent on detection of a positive IgM response and are insensitive for about 1-2 weeks of infection (Aguero-Rosenfield and Wang et al., 2005). After the first several weeks of infection, diagnosing Lyme disease generally relies on the observation of clinical symptoms in addition to a positive antibody response to *B. burgdorferi* tested by whole-cell sonicate ELISA and Western

Blot procedures (Aguero-Rosenfield and Wang et al., 2005). The results are then interpreted using the criteria provided by the Centers for Disease Control and Prevention (CDC, 1995) and the treatment guidelines are available from the Infectious Disease Society of America (Wormser, 2006).

Immunoglobulins

The immune system produces proteins called immunoglobulins, which bind to and neutralize foreign substances in the body. Immunoassays such as ELISA and Western Blot are based on the binding that occurs between an immunoglobulin (antibody) and a substance that it recognizes, called an antigen (Nowakowski and Schwartz et al., 2001). IgM antibodies to *B. burgdorferi* are the first response to the infection. They are made in great quantities and are pentavalent, whereas IgG antibodies are monomeric. IgM antibodies represent a new and active infection when they are present in large numbers. The number of IgM antibodies decreases as the active infection resolves (Goetnner and Schulte-Spechtel et al., 2005). However, in Lyme disease, IgM antibodies can also persist after the infection resolves. IgG antibodies, which are more specific, are made about a week later in the infection. An immunoassay allows for detection of a specific antibody or antigen in bodily fluids. When testing for the presence of an antibody, specific antigens are used to detect the antibodies in the sample. If the antibodies are present, then the antigens in the test will bind to the antibodies and with activation of an enzyme marker, the test will be positive. A secondary anti-human IgG (anti-human goat IgG) is linked to an enzyme and then a substrate is provided for the enzyme. To identify the presence of specific antigens in a sample, antibodies are

similarly used in the assay (Aguero-Rosenfield and Wang et al., 2005). The reaction given is then compared to reactions of known concentrations and the amount of antigen is calculated.

Enzyme-Linked Immunosorbent Assay (ELISA)

The ELISA is a common type of immunoassay used for the testing of Lyme disease. It is a test based on the binding of antigen-antibody complexes. In order to detect an antibody in a patient's blood, specific *B. burgdorferi* antigen(s) are attached to a solid surface of the plate. Afterwards, the patient sample is added. If the sample added contains the antibody that is being tested for, it will bind to the spirochetal antigen. Then, a secondary antibody, one that recognizes human antibodies, which is labeled with an enzyme, is added to the mixture. The secondary antibody that is commonly used when testing for Lyme disease is made in a goat. The enzyme linked to the secondary antibody creates a detectable color change when the enzyme's substrate is added (Aguero-Rosenfield and Wang et al., 2005). This usually creates various shades of yellow in the samples depending on how many of the antibodies are present. The antibody isotypes that are typically tested for in Lyme disease are IgM and IgG.

Western Blot

The Western Blot is a second test that is done in patients who have positive or equivocal results by ELISA. It detects antibodies in serum by combining electrophoresis with an additional step that blots proteins that have been separated onto a membrane. To begin performing a Western Blot, the sample containing spirochetal proteins is added to a

spot on one end of a gel strip. Then, an electric current is passed through the gel, which causes the proteins to separate according the size and shape, forming bands. The bands are then transferred onto a thin membrane that is put in contact with the gel plate (Aguero-Rosenfield and Wang et al., 2005). The presence of certain types of proteins is compared to a known control sample for interpretation of results. The 10 IgG proteins tested for in the CDC criteria are 18, 23, 28, 30, 39, 41, 45, 58, 66, and 93-kD.

Treatment

Treatment varies depending on the stage of disease. Patients with erythema migrans, the first stage of the infection, can usually be successfully treated with a 10-14 day course of either doxycycline, amoxicillin or cefuroxime axetil. Treating erythema migrans shortens the duration of the manifestation and prevents the next stages of Lyme disease (Steere, 2016). For those with cardiac or neurological symptoms, a similar approach is followed with a course of antibiotics ranging from 14-21 days. However, for those with Lyme arthritis, a late infection stage, a 28-day course of oral antibiotics is recommended. In those who continue to have joint inflammation following therapy, 14-28 days of intravenous antibiotic therapy is prescribed (Steere, 2016). For patients that do not respond to either oral or intravenous antibiotics (antibiotic refractory Lyme arthritis), NSAIDs or DMARDs are generally given. In most patients, the inflammation resolves within months, but in extreme circumstances, arthroscopic synovectomy is performed (Steere, 2016).

Autoantigens

Three autoantigens, ECGF, MMP-10 and ApoB-100, have been associated with Lyme disease, particularly Lyme arthritis. About 10-30% of patients with various manifestations of Lyme disease had T or B cell responses to each of the 3 autoantigens and most commonly those with antibiotic-refractory arthritis (Drouin, 2013; Crowley, 2015; Crowley, 2016). Moreover, patients with antibiotic refractory Lyme arthritis had significantly higher levels of ECGF and MMP-10 protein in joint fluid or synovial tissue than patients with antibiotic-responsive arthritis. Autoimmune involvement in the pathogenesis of Lyme may occur early and late in the disease. Later in the disease, in patients with antibiotic-refractory arthritis, who have marked antigen accumulation and excessive inflammation, the autoimmune response may become pathogenic. In the synovia of these patients, autoantibody responses may cause immune complex deposition and tissue damage, leading to fibrosis and obliterative microvascular lesions in the tissue (Drouin, 2013; Crowley, 2015; Crowley, 2016).

Th17 and Antibiotic Refractory LA

Even though these autoantibody responses appear to be nonpathogenic in the beginning stages of infection, they are associated with T cell responses and pathogenic joint inflammation in later stages of the disease (Strle, 2017). Th1 mediators are necessary for control of the infection, but excessive levels lead to a more severe disease outcome. Th17 responses also help to control early infection, but prolonged Th17 responses mat lead to autoimmunity and tissue pathology. In patients with antibiotic-refractory Lyme arthritis, Th17 responses correlate with autoantibody levels, suggesting

that Th17 responses are important in autoimmunity in patients with antibiotic-refractory Lyme arthritis (Strle, 2017).

Research Goals & Hypothesis

Lyme disease is a vector-borne illness caused by the spirochete *Borrelia burgdorferi*. The infection is epidemic in parts of the northeastern U.S. Although much research has been done, there is still much to learn about pathogenesis. Research has shown differences in IgG and IgM antibody levels in patients with Lyme disease depending on the stage of the infection. IgM antibodies, the initial antibody response, are prominent at the beginning of the infection and decline as time passes, while IgG antibodies, the more specific response, increase as the infection progresses. Knowing the variations in the levels of antibodies throughout the infection gives clinicians more accurate information on whether or not the patient has the disease and how much it has progressed. The strains of *B. burgdorferi* in the northeastern United States are particularly arthritogenic (Jones et al., 2009). In 1990, selective concentration of IgA antibodies to *B. burgdorferi* in cerebrospinal fluid was reported in patients with neuroborreliosis (Steere, 1990).

Little is known about IgA antibodies in Lyme disease. IgA antibodies are present primarily in mucosal areas of the body. Since Lyme disease does not involve mucosal sites, it is surprising that IgA antibody responses would be relevant in Lyme disease. Yet, earlier data has suggested that IgA antibody responses to *B. burgdorferi* may be present with certain manifestations of the infection. In our study, we determined IgA antibody responses to *B. burgdorferi* systematically in serum in patients with erythema migrans, neuroborreliosis, carditis, and in serum and joint fluid in patients with responsive and refractory Lyme arthritis. This should provide insights into disease pathogenesis and may also help create better diagnostic tools for the disease.

For this purpose, we will determine IgA antibody levels to *B. burgdorferi* and 3 Lyme disease-specific autoantigens (MMP-10, ECGF, and ApoB-100) by ELISA. The results derived from a microplate reader will then be graphed and analyzed accordingly to compare the results within and between groups. We hypothesize that by testing patients' serum and joint fluid IgA antibody levels to *B. burgdorferi* and 3 specific autoantigens in patients with various stages of Lyme disease, we will be able to determine whether IgA levels are significantly elevated at a given stage and whether IgA levels are elevated in a localized area of inflammation in joints. In particular, we wanted to determine whether IgA antibody responses were different in patients with antibiotic-response or antibiotic-refractory arthritis. If not, we would reject our hypothesis if there are no significant differences in IgA antibody levels between responsive or refractory patients or among those with various clinical manifestations of Lyme disease.

Implications of Research

This research was done with the hope that it would explain more about the pathogenesis of Lyme disease and immune phenomena in antibiotic-refractory Lyme arthritis. Additionally, we anticipate that there will be notable differences between IgA antibody levels in the serum and joint fluid of these patients. Understanding the variation among the responses between IgA to *B. burgdorferi* and 3 specific autoantigens will help foster our understanding of how the disease affects patients systemically. Moreover, we will be able to correlate cytokine levels with IgA antibody values and with disease duration, which will further out knowledge of antibiotic-refractory Lyme arthritis.

Definition of Terms

Periplasm: Concentrated matrix in the periplasmic space which is in between the inner cytoplasmic membrane and the bacterial outer membrane

Spirochete: Group of bacteria that are spiral-shaped, can be serious pathogens to humans and cause diseases such as Lyme disease and syphilis

Outer Surface Protein C (ospC): A helical major antigen on the surface of *Borrelia burgdorferi* during transition from the tick to the mammalian host

Erythema Migrans: An expanding skin lesion seen in early stages of Lyme disease as a result of a skin infection caused by the spirochete

Arthralgia: Pain and stiffness of the joints

Myalgia: A non-specific symptom of Lyme disease that results in muscle pain

Dissemination: Spread of infection from the skin to areas of the body other than where it originated

Lymphocytes: White blood cells that have three basic types: B cells, T cells, and NK (natural killer) cells

B cells: White blood cells that produce antibodies to attack invading bacteria and viruses

T cells: White blood cells that destroy body's own cells that have become cancerous or taken over by viruses and can help B cells to produce antibodies

Dendritic Cells: An antigen-presenting cell and activates T cells and stimulates growth and differentiation of B cells

Macrophages: Large white blood cells that detect, phagocytose, and destroy bacteria and harmful organisms

Innate Immune Response: Nonspecific defense mechanisms, such as, immune system cells or chemicals in blood that can respond immediately or within hours of an antigen appearing in the body

Adaptive Immune Response: Antigen-specific immune response that begins when an antigen is recognized but requires several days to create immune cells that specifically focus on attacking the antigen

Peripheral Blood Mononuclear Cells (PBMC): Peripheral blood cell with a round nucleus including lymphocytes and monocytes

Cytokines: Substances secreted by certain cells of the immune system that affect other cells (i.e. interferon, interleukin and growth factors)

Chapter II

Materials & Methods

ELISA Preparation Day 1

The purpose of the experiment is to measure the possible upregulation of IgA antibodies to *B. burgdorferi* or 3 Lyme disease-specific autoantigens in patients with various stages of Lyme disease. We studied this by testing serum and if available, joint fluid samples in patients with Lyme arthritis (responsive and refractory), and serum samples in patients with EM (erythema migrans), neuroborreliosis (meningitis/facial palsy), or carditis (Table 1). A microplate was coated with the respective antigen and left overnight. An ELISA was performed the next day to measure IgA antibody levels.

The experiment was performed as follows:

The first experimental group was patients with Lyme arthritis (LA patients). The first group of the plates was coated with *B. burgdorferi* antigens and patients' serum samples were tested for IgA responses. A 96-well plate was coated with 31µl of B67 (mixture used to coat a plate to test for *B. burgdorferi*) in 10.2 ml of coating buffer (100µl of the mixture in each of the 96 wells). The plate was left in the cold room overnight.

ELISA Day 2

The next day, the plate was washed x 3 with the rinsing buffer (Table 2) after which the plate was coated with 200µl of blocking buffer in each well (Table 2). After the coating, the plate was set on a shaker for 60 minutes. In the meantime, 2.5µl of each of the patient samples was transferred into different wells on a stamping plate. The stamping plate was diluted with 125µl of blocking buffer in each well that the patient sample had been transferred to. The plate also included blanks and serum samples from healthy controls. After 60 minutes, the plate was rinsed with a rinsing buffer x 3 and 100µl from the mixture of each of the patient samples on the stamping plate was pipetted into the microplate. This was then put on the shaker for 90 minutes. When 10 of the 90 minutes remained, a conjugate solution was prepared. 2.5µl of the IgA conjugate was added to 10ml of blocking buffer. After 90 minutes, the plate was washed x 3 with the rinsing buffer and 100µl of the conjugate mix was added to each of the wells and put on the shaker for another 90 minutes. When 20 minutes remained, TMB substrates A and B were mixed at a 1:1 ratio totaling 10.2 ml. After 90 minutes, the plate was rinsed x 5 with the rinsing buffer, and 100µl of the TMB was transferred into each of the 96 wells. The reaction was stopped after 6 minutes with the addition of 100µl of sulfuric acid into each well. From past experiments, 6 minutes had been shown to be the optimal time to stop the reaction. The substrate caused positive responses to turn shades of blue and sulfuric acid turned the responses into shades of yellow. The plate was then inserted into the microplate reader (set to 450nm for wavelength and a 5 second read), which quantified the results for each of the wells depending on the intensity. The values were then compared between the Lyme samples and controls to assess the significance.

The method was then repeated with the same dilutions and samples for each autoantigen (MMP-10, ECGF, and Apo-B) except for the difference in the coating buffer type and dilution.

Statistical Analysis

The figures were constructed using the GraphPad Prism program. For figure 1, the significant differences were calculated using the Fisher's epact test. The correlations were calculated using Pearson's correlation coefficient, and the values were adjusted using the Benjamini-Hochberg formula (i/m)Q.

Research Limitations

Inconsistent results by the ELISA might occur due to improper pipetting or contamination. To avoid this, we ran a row of duplicates on each plate to verify that the results were accurate. Running the duplicates showed us whether the identical samples had the same or similar result. In the event that there was variation among the results using the duplicate sample, we ran the test again the next day to solidify the results. Only one test was run the next day due to variation in the duplicates.

Chapter III

Results

IgA Antibodies to *B. burgdorferi* and Autoantigens

IgA antibody levels to *B. burgdorferi* and 3 Lyme disease-specific autoantigens were determined in serum samples from 188 patients with various manifestations of Lyme disease. In patients with antibiotic-responsive arthritis, serum samples were obtained prior to antibiotic treatment when patients were still infected. In contrast, serum samples in the refractory group were usually collected at the conclusion of antibiotic treatment when few if any live spirochetes remained.

As shown in figure 1, significant serum IgA antibody responses to the spirochete were seen in a minority of patients with each of the early or late manifestations of the illness. The highest frequencies and the highest levels of IgA antibodies to *B. burgdorferi* were found in joint fluid of patients with Lyme arthritis, a late disease manifestation. At this site, 66% of antibiotic-responsive patients had high IgA antibody responses, suggesting that they had localized production of anti-spirochetal IgA antibodies in infected joints. IgA antibody titers to *B. burgdorferi* tended to be lower in the refractory group, consistent with the idea that spirochetes had been killed with antibiotic therapy, even though joint inflammation persisted.

With respect to the 3 Lyme disease-associated autoantigens (ECGF, MMP-10, and apoB-100), the highest percentage of patients with elevated IgA antibody levels was to apoB-100 throughout the various manifestations of the illness. However, there were not significant differences in apoB-100 antibody levels between serum and joint fluid or between antibiotic-responsive and refractory Lyme arthritis patients. Instead, the IgA

antibody response to Apo-B in was significantly higher in patients with carditis. Apo-B is an important component of plaques that are involved in atherosclerotic cardiovascular disease (Daniels et al., 2009), but it is not yet known why IgA response to apoB-100 would be particularly elevated in patients with Lyme carditis.

As with apoB-100, IgA antibody responses to MMP-10 and ECGF were found primarily in serum (Figure 1). However, a few refractory patients had IgA responses to MMP-10 or ECGF in joint fluid whereas responsive patients did not. Thus, IgA antibody responses to *B. burgdorferi* were highest in joint fluid, while IgA reactivity to the autoantigens was greater in serum. This is illustrated in figure 2 with lines connecting serum and joint fluid values in each patient.

IgA Correlations with Cytokines

Various cytokines representative of innate, Th1, or Th17 immune responses were measured previously by Dr. Klemen Strle using the same serum and joint fluid samples tested here for IgA determinations. Among serum samples from 25 patients with erythema migrans, an early manifestation of the disease, there was a trend toward correlations between Th17 adaptive responses and IgA antibody responses to *B. burgdorferi* (Table 4). This suggests that as Th17 adaptive responses increase at the beginning of the disease, IgA antibody responses to *B. burgdorferi* also rise. There were no significant correlations between IgA autoantibody responses and cytokine levels.

IgA antibody responses to *B. burgdorferi* and the 3 autoantigens were then correlated with the levels of these cytokines in serum of 109 patients with Lyme arthritis, 46 of whom also had joint fluid available. The data were stratified into antibiotic-responsive and antibiotic-refractory groups. In serum, there were no correlations between *B. burgdorferi* IgA antibody values and any cytokine levels in either responsive or refractory patients (Table 5). With the autoantigens, the only significant correlation was between ECGF IgA antibody values and CXCL9 levels in the responsive group.

Similarly, in joint fluid, there were no significant correlations between IgA *B. burgdorferi* or autoantibody values and cytokine levels in either the responsive or refractory groups (Table 6). However, there was a trend toward an inverse correlation between IgA *B. burgdorferi* antibody values and Th17-associated cytokine levels in the refractory group. Thus, the lower the IgA *B. burgdorferi* antibody value, the higher the level of IL-17F, IL-23, IL-25, or IL-27.

This is interesting to compare to the correlations in serum in patients with erythema migrans to those in joint fluid in patients with antibiotic-refractory Lyme arthritis (Tables 5 and 6). There is a positive trend between the Th17cytokine responses and IgA antibody reactivity to *B. burgdorferi* in patients with erythema migrans, but a negative (inverse) trend between these factors in patients with antibiotic-refractory arthritis. This implies that IgA antibody responses and Th17 cytokines increase in the initial infection, but as the Borrelia response declines after antibiotic treatment in patients with refractory arthritis, the Th17 cytokines may be secreted in response to autoantigens.

IgA Correlations and Disease Duration

IgA antibody responses to *B. burgdorferi* and 3 specific autoantigens were correlated with duration of arthritis in 93 patients with antibiotic-responsive or antibiotic-refractory Lyme arthritis. In the refractory group, IgA antibody values in serum to each of the 3 autoantigens correlated with the duration of arthritis from onset to study entry, from study entry to resolution of arthritis (except for MMP-10), and with the total duration of arthritis (Table 7). Thus, the longer the duration of post-infectious, antibiotic-refractory Lyme arthritis, the higher the IgA antibody levels to each of the 3 autoantigens. There was no correlation between joint fluid IgA antibody values and duration of arthritis (Table 8). Similarly, there was no correlation between *B. burgdorferi* IgA antibody levels and arthritis duration in either serum or joint fluid.

Chapter IV

Discussion

Significance of IgA

Although IgG is the most abundant antibody circulating in serum, IgA is by far the most abundant isotype overall. More IgA is produced (66mg/kg/d), primarily in the gut mucosa, than all other classes of antibodies combined (Robert et al., 2013). However, most of the IgA produced is lost through the exocrine secretions due to extensive mucosal surfaces. Secretory IgA is synthesized as a dimer by local plasma cells and is transported to mucosal surfaces through epithelial cells by the polymeric Ig receptor (Monteiro et al., 2010). In contrast, serum IgA is usually in its monomeric form (85-90%) and is the second most abundant isotype in circulation. Most of the serum IgA is derived from bone marrow plasma cells.

There are two subclasses of IgA: IgA1 and IgA2. The subclasses of IgA differ by the absence of a 13-amino acid sequence in the hinge region of the IgA2 molecule (Macpherson et al., 2007). IgA1 is the predominant form of IgA found in serum, whereas secretory IgA (IgA2) is mostly present in mucosal surfaces, and it has strong anti-inflammatory properties (Holmgren et al., 2005). It is generally present in the polymeric form, which has 2 molecules of IgA joined together by a J-chain. The J-chain (additional 18kDa and 137 residue polypeptide chain) is comprised of 2 immunoglobulin-like domains. It's covalently attached to the C terminal Cys471 on IgA's Ch3 domain via a disulfide bridge with either the J chain's Cys 14 or Cys 68. The J chain increases rigidity

through a single N-linked oligosaccharide and allows the IgA to form dimers (Schroeder et al., 2010).

Human serum IgA has either anti-inflammatory or pro-inflammatory capacity, which likely contributes to feedback mechanisms maintaining a balance between pro-inflammatory and anti-inflammatory properties (Olas et al., 2005). In the absence of antigen, monomeric IgA in serum has an anti-inflammatory role (Papista et al., 2011). In this circumstance, serum monomeric IgA down-regulates IgG-mediated phagocytosis, chemotaxis, bactericidal activities, oxidative burst activity and cytokine release (Monteiro et al., 2010). This seems to be important in patients with selective IgA deficiency who have little or no IgA, and have increased susceptibility to autoimmune and allergic disorders including rheumatoid arthritis (Jacob et al., 2008).

In non-pathogenic environments, monomeric IgA binds to FcRγ-associated FcαRI and promotes the partial phosphorylation of FcRγ, which in turn, induces an inhibitory ITAM (immunoreceptor tyrosine-based activation motif) configuration that results in sequential recruitment of Syk (partial activation) followed by the recruitment of SHP-1 phosphatase (Ivashkiv et al., 2009). This process allows for cell inhibition such as inhibition of heterologous receptor activation by external agents (Monteiro et al., 2010).

However, when antigen is recognized, serum IgA interacts with the Fc receptor (FcαRI), which is expressed on immune effector cells and the interaction initiates inflammatory reactions (Snoeck et al., 2006). IgA immune complexes lead to enhanced IgA binding to the FcαRI receptor on blood myeloid cells (dendritic cells, neutrophils, eosinophils, monocytes/macrophages). Cell activation then occurs through the crosslinking of transmembrane FcRγ-associated FcαRI and the recruitment of Syk and

other activating effectors such as Src (Monteiro et al., 2010). This increases intracellular free Ca^{2+}, which results in the signaling cascades for pro-inflammatory cytokines (Ramadan et al., 2011).

Prior to antibiotic therapy, in patients with erythema migrans or Lyme arthritis, IgA antibodies to *B. burgdorferi* may have a pro-inflammatory role in control of the infection.

Role of FcαRI

IgA Fc receptor type 1 (FcαRI) plays a dual role in immunity. The receptor is expressed on blood myeloid cells including dendritic cells, neutrophils, eosinophils, and monocytes/macrophages (Monteiro et al., 2003). Although FcαRI plays an anti-inflammatory role in immunity under physiologic conditions (by following transmission of inhibitory signals following binding of serum IgA), it has inflammatory properties when under pathologic conditions or exacerbated immune responses (Pasquier et al., 2005). During this time, FcαRI is aggregated by IgA immune complexes, which leads to its activating state where it performs functions such as phagocytosis, cytokine release, antibody dependent cellular cytotoxicity, superoxide release and antigen presentation (Monteiro et al., 2003).

Whereas γ-less FcαRI recycles monomeric IgA and plays an essential role in mIgA homeostasis, FcRγ-associated FcαRI mediates either activating or inhibitory responses. This dual function receptor operates based on the type of ligand, multimer, or monomer (Pasquier et al., 2005). Both opposing functions are mediated by the FcRγ adaptor, which contains an immunoreceptor tyrosine-based activation motif (ITAM) in its

cytoplasmic tail. The configurations (activating versus inhibitory) are dependent on the way that the ITAM-containing receptor interacts with its ligand (Blank et al., 2009).

Immune Responses

The cytokines that we correlated with IgA antibody responses are involved in innate, Th1, or Th17 responses. Innate responses are responsible for immediate responses to an infection and recruit immune cells to the site of infection through the production of chemical factors, including cytokines, particularly TNF (Janeway et al., 2001). They act as a physical and chemical barrier to antigens and activate the complement cascade to identify pathogens and promote clearance of antibody complexes. The chemical factors released by innate responses are a source for inflammation at the site of infection and serve as a physical barrier against the spread of infection (Levine et al., 2011).

Th1 responses produce pro-inflammatory cytokines, particularly interferon gamma (IFNγ), which are responsible for killing intracellular bacteria or viruses and may sustain autoimmune responses. However, without appropriate control of these responses, the pro-inflammatory reaction can cause sustainable tissue damage (Berger et al., 2000). Anti-inflammatory responses, such as Th2, which include cytokines Il-4, 5, and 13, help to control excessive inflammation (Berger et al., 2000). However, in other circumstances, Th2 responses are pro-inflammatory and are important in controlling parasites and can be a pro-inflammatory response in asthma.

Th17 responses are initially important in providing rapid protection against extracellular pathogens. However, prolonged Th17 responses, with secretion of IL-23, may lead to autoimmunity and tissue injury (Bystrom et al., 2015). The receptors for Il-

17 are widely expressed on epithelial cells in the gut, which result in Th17 responses being great mediators between the immune system and tissues (Ouyang et al., 2008).

In Lyme disease, innate, Th1 and Th17 immune responses, which lead to IgM, IgG and IgA antibodies to *B. burgdorferi* are each important in the control of early infection. As shown here, Th17 responses correlated with IgA antibodies to *B. burgdorferi* early in the infection. In contrast, it has been shown previously that Th17 responses correlate with IgG levels of 3 Lyme disease-associated autoantibodies in patients with antibiotic-refractory arthritis (Strle, 2017). We add here that the levels of IgA antibodies to these 3 autoantigens correlated with the duration of arthritis in the refractory group.

Conclusions

In summary, IgA antibody responses to *B. burgdorferi* were highest in patients with Lyme arthritis. In patients with antibiotic-responsive arthritis, the arthritis group that was still infected when the samples were obtained, the values were greater in joint fluid than in serum, suggesting local production of IgA antibodies in synovial tissue during the infection. In contrast, IgA antibody responses to the 3 Lyme disease-associated autoantigens were similar to or greater in serum than in joint fluid, suggestive of production at extra-articular sites. However, the 3 autoantigens differed in when responses were greatest. With apoB-100, they were greatest in patients with carditis; with MMP-10, they were greatest in patients with Lyme arthritis, and with ECGF, they were similar throughout the disease.

Correlations of IgA antibody responses with cytokine profiles showed that IgA antibody responses to *B. burgdorferi* in serum tended to correlate directly with Th17-associated cytokines early in the infection in patients with erythema migrans. In contrast, there was a trend toward inverse correlations between IgA antibodies to *B. burgdorferi* and Th17-associated cytokines in joint fluid in patients with antibiotic-refractory arthritis who were usually seen after antibiotic-treatment when few, if any, spirochetes remained. Finally, in the refractory group, IgA antibody responses to each of the 3 autoantigens correlated with the total duration of arthritis, whereas IgA antibody responses to *B. burgdorferi* did not show this correlation, suggesting that immune reactivity to the autoantigens plays a role in the pathogenesis of antibiotic-refractory arthritis.

These findings lead us to accept our hypothesis that IgA levels to *B. burgdorferi* are highest in patients with Lyme arthritis, and the responses to *B. burgdorferi* and the 3

autoantigens vary between patients with antibiotic-responsive or antibiotic-refractory arthritis. Moreover, these data provide clues to disease pathogenesis. In most instances, IgA antibody responses to *B. burgdorferi* appear to help control the infection, but the IgA responses to the 3 Lyme disease-associated autoantigens may be disadvantageous in those with refractory arthritis, leading to a longer duration of joint inflammation.

Future Directions

To further research the implications of IgA antibody responses in Lyme disease, a Western Blot could be done to identify specific spirochetal proteins that are targeted by IgA antibodies in Lyme disease. Comparisons between IgG and IgA Western Blot could potentially lead to a greater understanding of why an IgA response is generated. In addition, knowing the role of IgA responses to autoantigens in Lyme disease could help us understand whether these autoantigens have a pro-inflammatory or anti-inflammatory response in antibiotic-refractory Lyme arthritis.

Appendix

Figure 1: IgA responses to *Borrelia Burgdorferi* and autoantigens in Lyme patients with different manifestations of the disease.

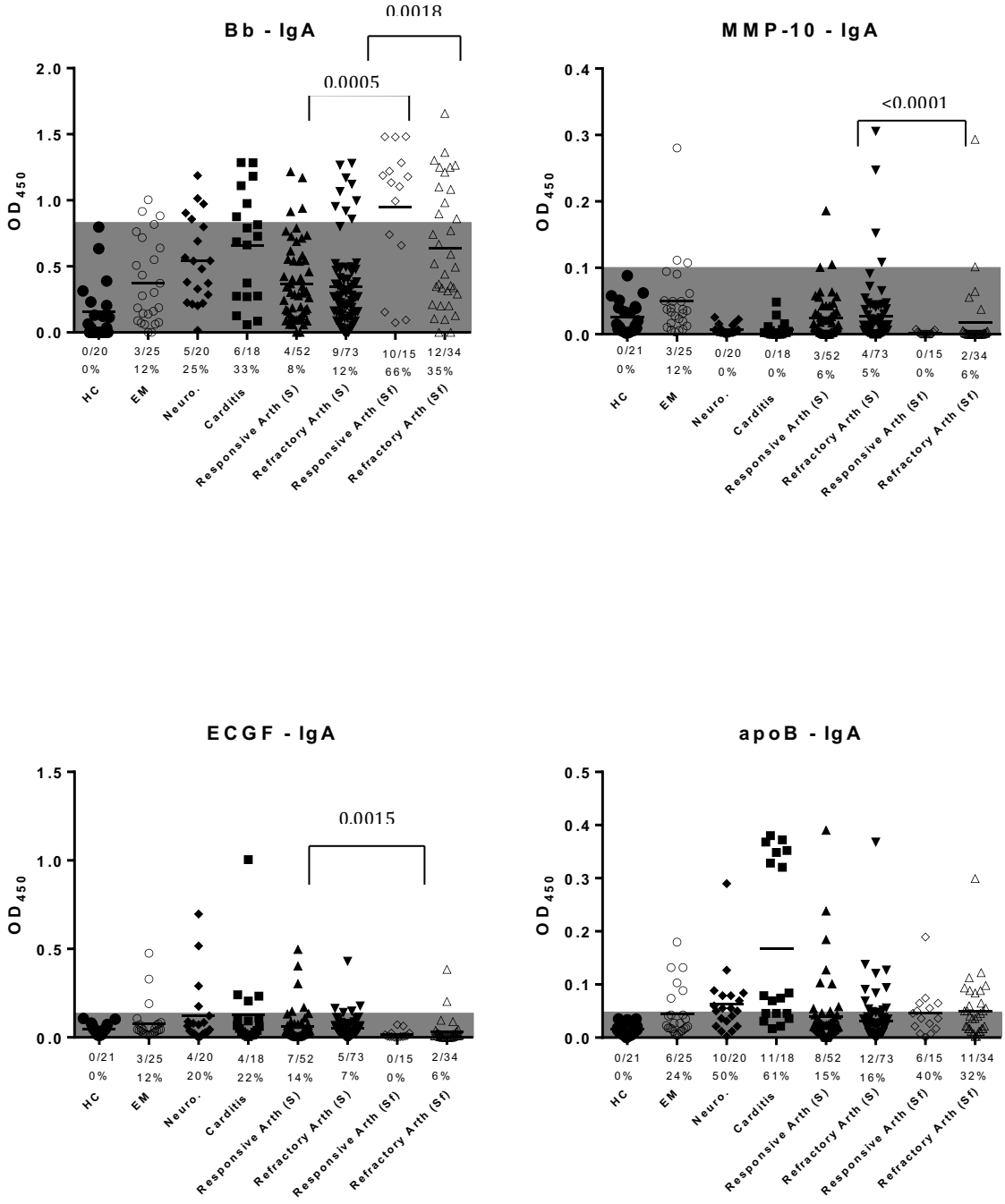

Figure 2: Comparison of the responses between serum and joint fluid of patients with Lyme arthritis.

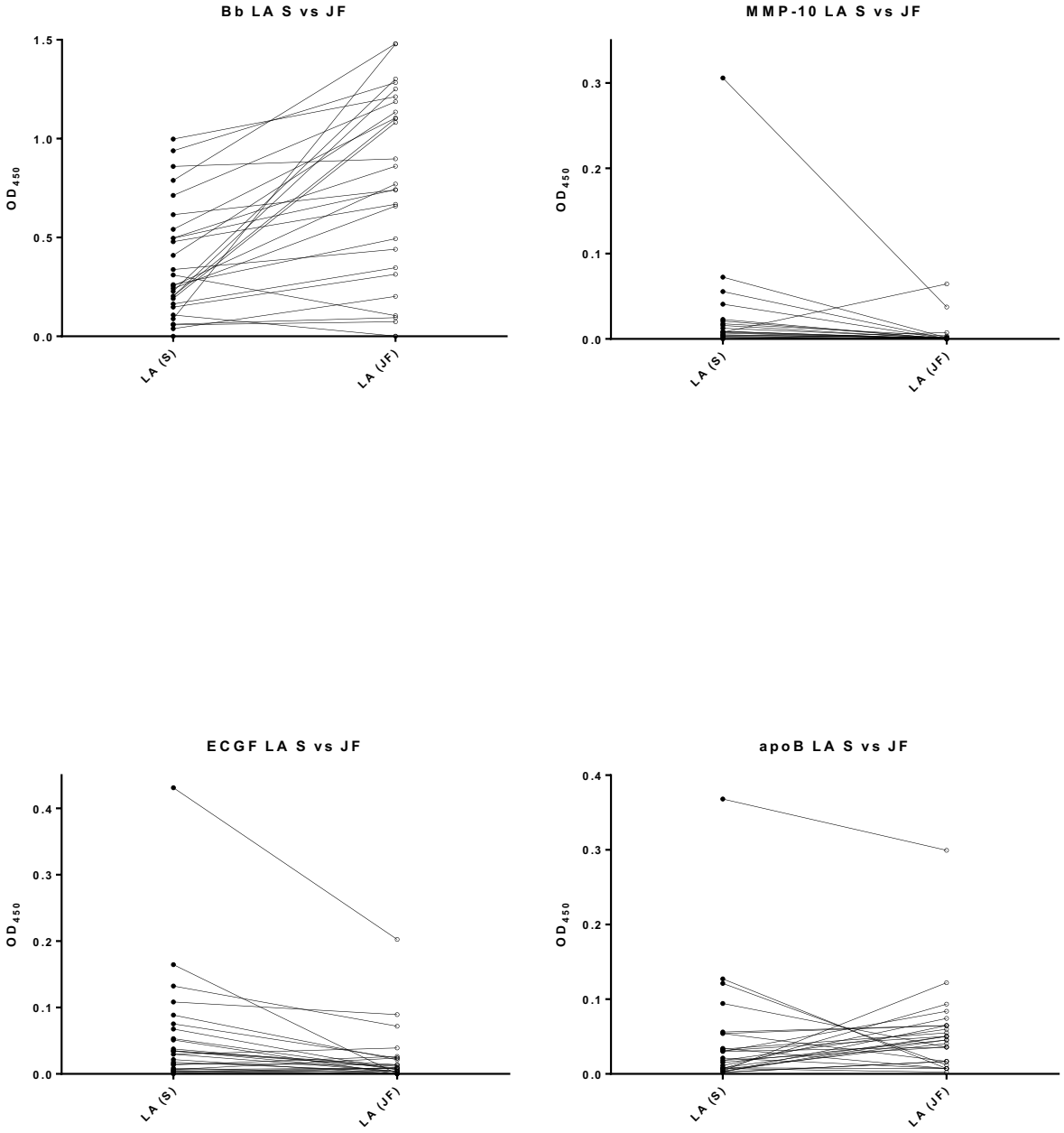

Table 1: Type of ELISAs run on various patient samples

Patient Sample Type (Dilution 1:100)	**ELISA IgA Antibodies**
LA (Lyme arthritis)	*B*orrelia *Burgdorferi*
JF (Joint Fluid)	MMP-10
Neuroborreliosis	ECGF
EM (erythema migrans)	Apo-B
Post Lyme	
Carditis	

Table 2: ELISA buffer recipes (coating buffer, blocking buffer, rinsing buffer)

ELISA Buffer Recipes		
Coating Buffer	Blocking Buffer	Rinsing Buffer
(5 omM Carbonate, pH 9.6) For 500ml: Na_2CO_3 - 0.8g $NaHCO_3$ - 1.46g dH_2O - 500 ml *add 1N NaOH to pH 9.6	(1 x PBS, 0.05% Tween 20, 5% nonfat milk, pH 7.6) For ~ 400 ml: Milk - 25g 10x PBS - 50 ml dH_2O - 350 ml 1N NaOH - 3.6 ml Tween - 250µl	(1 x PBS, 0.05% Tween 20, pH 7.6) For 1000 ml: 10 x PBS - 100 ml dH_2O - 900 ml Tween 500µl *add 1N NaOH to pH 7.6

Table 3: Initial concentration vs. dilution of antigens

Antigen	**Initial Concentration**	**Dilution**
MMP-10	2.5mg/ml	4.08µl in 10.2 ml coating buffer
ECGF	100µg/ml	102µl in 10.2 ml coating buffer
Apo-B	0.5mg/ml	10.2µl in 10.2 ml coating buffer
Borrelia burgdoferi (B67)	4100µg/µl	31µl in 10.2 ml coating buffer

Table 4: Correlation of inflammatory mediators with IgA antibody responses to 3 Lyme disease associated autoantigens in serum of patients with erythema migrans

	Erythema Migrans (N=25)			
	Bb	ECGF	MMP-10	apoB-100
Innate Responses				
CCL2	<-0.1	<0.1	<-0.1	0.4
	0.6	0.9	0.9	0.05
CCL3	<0.1	-0.2	-0.1	0.3
	0.9	0.4	0.4	0.1
IL-1β	<0.1	0.4	0.2	0.5
	0.6	0.07	0.2	0.01
IL-6	0.2	0.3	0.1	0.4
	0.3	0.2	0.6	0.05
IL-8	-0.2	-0.1	<-0.1	0.2
	0.3	0.6	0.9	0.2
IL-10	<0.1	0.1	<-0.1	<0.1
	0.8	0.4	0.9	0.9
TNF	<0.1	-0.2	-0.2	0.1
	0.8	0.3	0.2	0.7
IFNα	0.2	-0.2	<-0.1	0.2
	0.3	0.3	0.7	0.4
Th1 Adaptive Responses				
IFNγ	0.2	-0.1	-0.1	<-0.1
	0.4	0.3	0.5	0.7
CXCL9	0.2	-0.2	-0.3	-0.2
	0.3	0.4	0.2	0.3
CXCL10	0.2	-0.2	-0.3	-0.1
	0.2	0.3	0.1	0.6
IL-12p40	0.3	0.4	0.1	0.2
	0.2	0.05	0.4	0.3
IL-12p70	0.2	0.1	-0.1	0.2
	0.3	0.7	0.6	0.3
CCL19	-0.1	-0.1	<-0.1	0.3
	0.4	0.5	0.8	0.2
Th17 Adaptive Responses				
IL-17A	0.5	<-0.1	<0.1	0.3
	0.01	0.9	0.8	0.1
IL-17F	0.4	<0.1	<-0.1	0.2
	0.01	0.9	0.9	0.3
IL-23	0.4	<-0.1	<-0.1	<0.1
	0.02	0.8	0.8	0.8
IL-25	0.4	<-0.1	<-0.1	0.1
	0.02	0.8	0.9	0.7
IL-27	0.4	-0.8	<-0.1	0.1
	0.02	0.6	0.7	0.6

*Correlations were performed using a Pearson correlation test. There was a trend toward correlation between IgA antibody values and Th17-associated cytokine levels. However, these correlations were not statistically significant based on $P \leq 0.05$ with Benjamini-Hochberg correction for multiple comparisons (false discovery date ≤ 0.1) and contain "*r*" and "*P*" label.

Table 5: Correlation of inflammatory mediators with IgA antibody responses to 3 Lyme disease associated autoantigens in serum of patients with antibiotic-responsive and antibiotic-refractory Lyme arthritis

	Antibiotic-Responsive LA (N=53)				Antibiotic-Refractory LA (N=56)			
	Bb	ECGF	MMP-10	apoB-100	Bb	ECGF	MMP-10	apoB-100
Innate Responses								
CCL2	<-0.1	-0.1	R=-0.3	R=-0.2	<-0.1	<-0.1	<0.1	<0.1
	0.8	0.3	P=0.04	P=0.09	0.9	0.9	0.9	0.9
CCL3	-0.1	R=-0.2	-0.1	-0.1	-0.3	-0.1	-0.1	<-0.1
	0.5	P=0.08	0.4	0.4	0.1	0.5	0.5	0.6
IL-1β	0.1	<0.1	R=0.4	<-0.1	<-0.1	-0.1	<-0.1	-0.1
	0.5	0.9	P=0.01	0.6	0.6	0.6	0.7	0.5
IL-6	<-0.1	<0.1	-0.1	<-0.1	-0.1	<0.1	<-0.1	<-0.1
	0.8	0.9	0.5	0.6	0.4	0.9	0.9	0.9
IL-8	<-0.1	0.3	0.3	0.2	<-0.1	<0.1	0.2	0.1
	0.9	0.1	0.2	0.2	0.7	0.7	0.4	0.5
IL-10	<0.1	<0.1	-0.2	<-0.1	<-0.1	<-0.1	<-0.1	<-0.1
	0.8	0.9	0.3	0.9	0.7	0.6	0.8	0.6
TNF	-0.3	0.1	0.2	<0.1	-0.2	0.1	<0.1	<-0.1
	0.02	0.4	0.2	0.6	0.2	0.5	0.9	0.8
IFNα	<-0.1	R=0.4	R=0.3	R=0.3	-0.2	-0.1	-0.1	<-0.1
	0.7	P=0.01	P=0.07	P=0.02	0.2	0.5	0.5	0.6
TH1 Adaptive Responses								
IFNγ	-0.1	<-0.1	<0.1	<-0.1	-0.1	0.2	<0.1	0.2
	0.6	0.8	0.6	0.8	0.4	0.3	0.9	0.3
CXCL9	<-0.1	**R=0.5**	<0.1	0.2	-0.1	<0.1	<0.1	<0.1
	0.8	**P=0.0003**	0.9	0.2	0.5	0.9	0.7	0.7
CXCL10	0.1	R=0.4	0.1	R=0.3	-0.1	0.1	0.1	0.1
	0.5	P=0.01	0.4	P=0.06	0.5	0.4	0.5	0.4
IL-12p40	0.1	<0.1	-0.2	<-0.1	-0.1	<-0.1	<-0.1	-0.1
	0.6	0.9	0.3	0.8	0.4	0.6	0.7	0.6
IL-12p70	-0.1	-0.2	-0.2	<-0.1	-0.2	-0.1	<-0.1	<-0.1
	0.4	0.4	0.4	0.7	0.2	0.5	0.6	0.6
CCL19	<0.1	<-0.1	R=0.4	<0.1	<-0.1	<-0.1	<0.1	<0.1
	0.9	0.9	P=0.002	0.8	0.7	0.8	0.7	0.9
TH17 Adaptive Responses								
IL-17A	-0.2	<-0.1	-0.1	<-0.1	-0.1	0.2	<0.1	0.2
	0.4	0.6	0.5	0.8	0.4	0.2	0.7	0.2
IL-17F	<-0.1	<-0.1	0.2	-0.1	-0.3	-0.2	<-0.1	-0.1
	0.9	0.8	0.2	0.5	<0.1	0.4	0.8	0.5
IL-23	0.2	<0.1	0.3	0.1	-0.2	-0.2	-0.1	-0.1
	0.2	0.8	0.1	0.6	0.3	0.4	0.6	0.6
IL-25	0.2	<0.1	0.2	0.1	-0.1	-0.1	<-0.1	<-0.1
	0.3	0.7	0.4	0.6	0.5	0.6	0.6	0.8
IL-27	0.1	<0.1	-0.2	<-0.1	-0.3	-0.1	<-0.1	<-0.1
	0.5	0.7	0.3	0.7	0.2	0.5	0.7	0.8

*Correlations were performed using a Pearson correlation test. Values in bold are statistically significant correlations based on $P \leq 0.05$ with Benjamini-Hochberg correction for multiple comparisons (false discovery date ≤ 0.1) and contain "*r*" and "*P*" label.

Table 6: Correlation of inflammatory mediators with IgA antibody responses to 3 Lyme disease associated autoantigens in the joint fluid of patients with antibiotic-responsive and antibiotic-refractory Lyme arthritis

	Antibiotic-Responsive LA (N=14)				Antibiotic-Refractory LA (N=32)			
	Bb	ECGF	MMP-10	apoB-100	Bb	ECGF	MMP-10	apoB-100
Innate Responses								
CCL2	R=-0.5	R=0.6	-0.2	<0.1	<-0.1	<-0.1	<0.1	<0.1
	P=0.09	P=0.03	0.5	0.9	0.9	0.7	0.9	0.8
CCL3	<0.1	-0.1	-0.2	<0.1	<-0.1	<-0.1	<-0.1	0.1
	0.9	0.6	0.5	0.9	0.9	0.9	0.6	0.5
IL-1β	<0.1	-0.1	<0.1	-0.1	<-0.1	-0.1	-0.2	-0.1
	0.9	0.7	0.9	0.7	0.7	0.6	0.3	0.5
IL-6	<-0.1	<0.1	-0.2	<0.1	-0.1	<-0.1	0.1	0.3
	0.9	0.9	0.4	0.8	0.6	0.7	0.6	0.1
IL-8	-0.2	0.1	-0.3	<0.1	0.2	0.1	<-0.1	-0.2
	0.56	0.7	0.4	0.9	0.3	0.6	0.9	0.4
IL-10	0.2	-0.1	R=-0.6	-0.3	<-0.1	0.3	-0.2	-0.3
	0.5	0.7	P=0.06	0.3	0.9	0.2	0.4	0.2
TNF	-0.3	0.4	-0.4	<0.1	-0.1	<-0.1	-0.2	<0.1
	0.3	0.2	0.2	0.9	0.6	0.9	0.3	0.8
IFNα	<0.1	0.2	0.2	-0.2	-0.2	R=0.4	-0.1	-0.1
	0.9	0.6	0.6	0.6	0.3	P=0.03	0.5	0.6
TH1 Adaptive Responses								
IFNγ	-0.4	R=0.5	-0.2	-0.1	-0.2	-0.1	-0.2	<-0.1
	0.2	P=0.09	0.6	0.7	0.3	0.4	0.3	0.9
CXCL9	<-0.1	R=0.5	-0.5	<0.1	-0.1	<-0.1	<0.1	<-0.1
	0.5	P=0.08	0.1	0.8	0.5	0.9	0.8	0.7
CXCL10	0.3	-0.3	-0.4	0.4	<-0.1	-0.1	-0.3	-0.2
	0.3	0.4	0.2	0.2	0.9	0.5	0.2	0.4
IL-12p40	0.2	<-0.1	-0.1	-0.2	-0.2	<-0.1	-0.3	-0.3
	0.5	0.9	0.7	0.5	0.4	0.8	0.2	0.1
IL-12p70	R=-0.6	R=0.6	-0.3	-0.1	-0.3	-0.1	<-0.1	<-0.1
	P=0.04	P=0.03	0.4	0.7	0.1	0.6	0.7	0.9
CCL19	-0.2	R=0.5	-0.4	<-0.1	-0.1	R=0.3	-0.2	0.1
	0.6	P=0.07	0.2	0.8	0.6	P=0.08	0.4	0.6
TH17 Adaptive Responses								
IL-17A	0.3	-0.3	-0.4	<-0.1	<-0.1	-0.2	-0.2	<-0.1
	0.3	0.3	0.2	0.8	0.8	0.2	0.3	0.8
IL-17F	-0.2	<0.1	0.3	-0.2	R=-0.4	-0.2	-0.1	<-0.1
	0.6	0.8	0.3	0.6	P=0.04	0.5	0.6	0.7
IL-23	<-0.1	0.2	0.2	<0.1	R=-0.4	-0.2	-0.1	<-0.1
	0.9	0.5	0.6	0.9	P=0.04	0.5	0.5	0.8
IL-25	-	-	-	-	R=-0.3	-0.1	-0.1	<-0.1
	-	-	-	-	P=0.08	0.5	0.6	0.8
IL-27	R=-0.7	0.4	-0.4	<-0.1	R=-0.4	-0.1	-0.1	<-0.1
	P=0.01	0.2	0.2	0.9	P=0.03	0.5	0.6	0.7

*Correlations were performed using a Pearson correlation test. Values in bold are statistically significant correlations based on $P \leq 0.05$ with Benjamini-Hochberg correction for multiple comparisons (false discovery date ≤ 0.1) and contain "*r*" and "*P*" label.

Table 7 (N=93): Clinical correlations with IgA antibody response to *B. burgdorferi* and 3 Lyme disease associated autoantigens in joint fluid of patients with antibiotic-responsive and antibiotic-refractory Lyme arthritis

	IgA (Responsive)				IgA (Refractory)			
	Bb	ECGF	MMP-10	apoB-100	Bb	ECGF	MMP-10	apoB-100
Age	-0.1 0.6	0.1 0.7	<0.1 0.9	-0.2 0.4	0.2 0.3	0.2 0.3	0.3 0.1	0 1
Duration arthritis onset to study entry (mos)	-0.4 0.2	0.2 0.5	0.1 0.7	-0.5 0.09	<0.1 0.8	0.4 0.1	0.4 0.07	0.3 0.2
Duration study entry to resolution (mos)	0.1 0.7	<0.1 0.8	<0.1 0.9	-0.2 0.6	<-0.1 0.9	0.2 0.4	0.3 0.3	0.2 0.4
Total Duration of Arthritis (mos)	-0.4 0.3	0.2 0.6	0.1 0.8	-0.5 0.1	<0.1 0.9	0.4 0.1	0.5 0.1	0.3 0.2

Table 8 (N=93): Clinical correlations with IgA antibody response to *B. burgdorferi* and 3 Lyme disease associated autoantigens in serum of patients with antibiotic-responsive and antibiotic-refractory Lyme arthritis

	IgA (Responsive)				IgA (Refractory)			
	Bb	ECGF	MMP-10	apoB-100	Bb	ECGF	MMP-10	apoB-100
Age	0.1 0.3	0.2 0.2	<-0.1 0.8	0.1 0.5	0.1 0.5	<0.1 0.6	<0.1 0.8	<0.1 0.9
Duration arthritis onset to study entry (mos)	<0.1 0.8	<0.1 0.7	0.1 0.5	<0.1 0.6	0.2 0.4	**R=0.5** **P=0.005**	**R=0.4** **P=0.01**	**R=0.4** **P=0.02**
Duration study entry to resolution (mos)	0.2 0.3	0.2 0.4	<0.1 0.7	0.2 0.3	<-0.1 0.9	**R=0.5** **P=0.008**	0.3 0.06	**R=0.4** **P=0.01**
Total Duration of Arthritis (mos)	<0.1 0.9	<-0.1 0.8	-0.1 0.5	-0.1 0.5	<0.1 0.7	**R=0.6** **P=0.0004**	**R=0.5** **P=0.006**	**R=0.5** **P=0.004**

*Correlations were performed using a Pearson correlation test. Values in bold are statistically significant correlations based on $P \leq 0.05$ with Benjamini-Hochberg correction for multiple comparisons (false discovery date ≤ 0.1) and contain "*r*" and "*P*" label.

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