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T_H17 Cytokine Responses in Lyme Disease Correlate with *Borrelia burgdorferi* Antibodies During Early Infection in Patients with Erythema Migrans and with Autoantibodies Late in the Illness in Patients with Antibiotic-Refractory Lyme Arthritis

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Running Title: T_H17 Responses in Lyme Disease

Summary

Patients with Lyme disease often have pronounced T_H17 inflammatory responses. Early in the infection, during erythema migrans, T_H17 responses correlate with *Borrelia burgdorferi* antibodies, whereas late in the disease, in patients with antibiotic-refractory Lyme arthritis, these responses correlate with autoantibodies.

Meeting information

Part of this work was presented at the American College of Rheumatology Annual Meeting in Boston MA, November 15-19, 2014.

ABSTRACT

Background: Control of Lyme disease is attributed predominantly to innate and adaptive T_H1 immune responses, whereas the role of T_H17 responses is less clear. Here we characterized these inflammatory responses in patients with erythema migrans (EM) or Lyme arthritis (LA) to elucidate their role early and late in the infection.

Methods: Levels of 21 cytokines and chemokines, representative of innate, T_H1, and T_H17 immune responses, were assessed by Luminex in acute and convalescent sera from 91 EM patients, in serum and synovial fluid from 141 LA patients, and in serum from 57 healthy subjects. Antibodies to *B. burgdorferi* or autoantigens were measured by ELISA.

Results: Compared with healthy subjects, EM patients had significantly higher levels of innate, T_H1, and T_H17-associated mediators ($P \leq 0.05$) in serum. In these patients, the levels of inflammatory mediators, particularly T_H17-associated cytokines, correlated directly with *B. burgdorferi* IgG antibodies ($P \leq 0.02$), suggesting a beneficial role for these responses in control of early infection. Late in the disease, in patients with LA, innate and T_H1-associated mediators were often >10-fold higher in synovial fluid than serum. In contrast, the levels of T_H17-associated mediators were more variable, but correlated strongly with autoantibodies to ECGF, MMP-10, and apoB-100 in joints of patients with antibiotic-refractory LA, implying a shift in T_H17 responses towards an autoimmune phenotype.

Conclusions: Patients with Lyme disease often develop pronounced T_H17 immune responses that may help control early infection. However, late in the disease, excessive T_H17 responses may be disadvantageous by contributing to autoimmune responses associated with antibiotic-refractory LA.

Keywords: Lyme disease, erythema migrans, Lyme arthritis, T_H17, antibodies.

T_H17 immune responses are important in the control of extracellular pathogens, but may also lead to autoimmune responses [1-4]. Exposure to IL-23 plays a critical role in the generation of pathogenic T_H17 cells [1, 2]. Such responses, particularly in patients with polymorphisms in the *IL23R* gene, have been implicated in the pathogenesis of several rheumatic diseases, including rheumatoid arthritis (RA), psoriatic arthritis, and ankylosing spondylitis [1, 2, 5-8].

Lyme disease is caused by the tick-transmitted spirochete, *Borrelia burgdorferi*, a large extracellular pathogen [9, 10]. In untreated patients, the infection usually occurs in stages with different manifestations at each stage [11-13]. The initial sign of the infection is usually an expanding skin lesion, erythema migrans (EM), which is often accompanied by flu-like symptoms such as myalgias, arthralgias, malaise, fever, or fatigue [11-13]. Months after disease onset, within the context of an expanded immune response to the spirochete, approximately 60% of untreated patients in the northeastern United States (US) develop arthritis in one or a few large joints, most commonly the knee [14].

Most Lyme arthritis (LA) patients respond well to oral and/or IV antibiotic therapy and their arthritis resolves, called antibiotic-responsive LA. However, a subset of patients have persistent proliferative synovitis for months to years after treatment with 2-3 months of oral and IV antibiotics and apparent spirochetal killing, termed antibiotic-refractory LA [15, 16]. Infection-induced autoimmunity is thought to be a contributing factor in this outcome [17-21]. To date, we have identified four autoantigens, endothelial cell growth factor (ECGF) [19], apolipoprotein B-100 (apoB-100) [18], annexin A2 [20], and matrix-metalloproteinase-10 (MMP-10) [17], that are each targets of T and B cell responses in ~10-35% of LA patients. Although antibodies to these autoantigens may appear early in the infection, they seem to be non-pathogenic at this stage, with little or no associated T cell responses. However, late in the disease, these autoantibodies are often accompanied by T cell responses, particularly in patients with antibiotic-refractory LA, in whom they are associated with specific pathologic findings in joints [17-20, 22].

Control of the *B. burgdorferi* infection in humans is attributed predominantly to innate and adaptive T_H1 immune responses. However, excessive levels of inflammatory mediators, particularly those linked to T_H1

responses, are associated with more severe disease, including more symptomatic early infection and antibiotic-refractory LA [23-25]. Although initial studies in animals and humans suggest a role for T_H17 immunity in Lyme disease and in post-Lyme disease sequelae [26-30], these responses are incompletely characterized, particularly in human disease.

We have previously analyzed innate and T_H1 adaptive immune responses in patients with early or late manifestations of Lyme disease and in tissue cell culture systems [23-25, 30-33]. Here we extend this work by characterizing T_H17 immune responses in patients with Lyme disease. We found that a subset of patients have pronounced T_H17 responses, which are associated with *B. burgdorferi* antibodies during early infection in patients with EM, and with autoantibodies in patients with LA, a late disease manifestation.

PATIENTS AND METHODS

Study patients

All patients met the Centers for Disease Control (CDC) criteria for the diagnosis of Lyme disease [34], and were treated according to the guidelines recommended by the Infectious Diseases Society of America [35]. All patients provided written informed consent. Human Investigation Committees at Tufts Medical Center (1988-2002) and Massachusetts General Hospital (2002-2016) approved the study.

For analysis of early infection, acute and convalescent serum samples and clinical information were available from 91 EM patients from the northeastern US seen between 1998 and 2001, all of whom were culture-positive for *B. burgdorferi*. In addition, serum, and in most instances synovial fluid samples were available from 141 patients with LA who were referred to the Rheumatology Clinic at Tufts Medical Center or Massachusetts General Hospital from 1988 through 2014. These patients, were referred before, during, or after antibiotic treatment. For comparison, serum samples were obtained from 57 healthy laboratory and hospital donors. Although subsets of these samples were used in previous studies of innate and adaptive T_H1 responses

[23-25, 32], all of the samples were tested again here, along with new samples, so that variability between assays would not be a factor in data interpretation.

Laboratory determinations

The levels of 21 cytokines and chemokines associated with innate (CCL2, CCL3, IL-1β, IL-6, IL-8, IL-10, TNF, IFNα) and adaptive T_H1 (IFNγ, CXCL9, CXCL10, IL-12p40, IL-12p70, CCL19), or T_H17 (IL-17A, IL-17F, IL-17E/IL-25, IL-21, IL-22, IL-23, IL-27) immune responses were assessed in acute and convalescent serum samples from EM patients, in serum and synovial fluid samples from LA patients, and in serum of healthy subjects using Luminex multiplex assays (EMD-Millipore). Because patient sample volumes were limited, cytokine and chemokine levels in these samples were determined once.

Antibody responses (IgG) to *B. burgdorferi*, or to 3 human autoantigens specific for Lyme disease (ECGF, apoB-100, and MMP-10), were determined for this study in ~30-50 patients with EM or LA as previously described [17-20].

Statistics

Differences in cytokine and chemokine levels were assessed using Mann-Whitney rank sum test. To show the range of values, differences among groups stratified according to EM-associated symptoms, or antibiotic-responsive versus antibiotic-refractory LA, were presented as box plots. Correlations involving inflammatory mediators and antibody responses were assessed using Pearson correlation test with Benjamini-Hochberg correction for multiple comparisons. Analyses were performed using SigmaStat software (SPSS). A P value ≤ 0.05 with FDR≤ 0.1 was considered statistically significant.

RESULTS

Inflammatory responses in patients with erythema migrans.

At study entry prior to antibiotic therapy, a median of 4 days after disease onset, the 91 *B. burgdorferi* culture-positive EM patients had significantly higher levels of 19 of the 21 mediators tested, including all T_H17 mediators (IL-23, IL-27, IL-25, IL-22, IL-17F, IL-21, and IL-17A) compared with healthy subjects. Representative mediators of each type of immune response are shown in Figure 1A. During acute EM, most patients had robust innate and T_H1 adaptive immune responses, with especially high levels of CCL2, CXCL9, and CXCL10, which are important chemoattractants for macrophages and CD4+ T-effector cells. Similarly, the levels of all T_H17 mediators assessed were greater in patients than in healthy subjects, although the values among patients were quite variable. The most highly expressed T_H17 mediator was IL-23. A subset of patients had both elevated T_H1 and T_H17 cytokine responses, but most had either high T_H1 or T_H17 responses, suggesting that some patients polarize towards a T_H1 or T_H17 immune response.

Three weeks later, during the convalescent period, soon after the conclusion of antibiotic therapy, the levels of T_H1-associated mediators had decreased dramatically, and in some cases approached the levels in healthy subjects (Figure 1A). Similarly, the levels of most innate mediators had decreased significantly during convalescence. In contrast, the levels of T_H17 mediators declined less during convalescence, suggesting the potential for persistence of these responses after spirochetal killing [30].

Of the 91 EM patients, 70 (77%) had at least one associated symptom, including fatigue, malaise, headache, fever, neck stiffness, arthralgias, chills, or myalgias. Patients with no symptoms had the lowest levels of inflammatory mediators; those with moderate symptoms (1-5 symptoms) had intermediate levels, and those with many symptoms (6-20 symptoms) had the highest levels (Figure 1B). The greatest differences among groups were observed for the T_H1-associated mediators, IFNγ, CXCL9, and CXCL10, and for the innate mediators, IL-8, TNF, and IL-6. Similar trends were observed for T_H17 mediators, but these did not reach statistical significance. Thus, appropriate innate and adaptive immune responses appear important for control of the infection, but the consequence of excessive inflammation is more symptomatic early infection.

Inflammatory responses in patients with Lyme arthritis.

In patients with LA, which usually occurs months after initial spirochetal exposure, the disease was generally confined to one or a few large joints, particularly the knee. In these patients, the inflammatory responses, particularly innate and T_H1 responses were concentrated in synovial fluid of the affected joints (Figure 2A). The levels of IFNγ-inducible chemokines, CXCL9 and CXCL10, which are potent chemoattractants for CD4+ T_H1-effector cells, were often ~100-fold higher in synovial fluid compared with serum ($P<0.001$). In addition, synovial fluid in LA patients contained very high levels of several innate immune mediators, including CCL2, IL-8, and IL-6. The levels of T_H17-associated mediators were also higher in LA patients compared with healthy subjects, but these responses were not uniformly concentrated in synovial fluid, suggesting that in some patients they may be occurring at extra-articular sites. Whereas the levels of IL-23, IL-27, and IL-17A were higher in synovial fluid compared with serum, the levels of IL-25, IL-22, and IL-17F were similar at both sites.

When LA patients were subdivided into those with antibiotic-responsive arthritis or antibiotic-refractory arthritis, the refractory group had significantly higher synovial fluid levels of most innate-associated mediators, and higher levels of the T_H1-associated mediators, CXCL9, CXCL10, and IFNγ (Figure 2B). In contrast, the levels of T_H17 mediators in synovial fluid were similar in both the responsive and refractory groups. In serum, the generally low levels of innate and T_H1-associated mediators were similar in patients with responsive or refractory arthritis, although there was a trend towards higher levels of T_H17-associated mediators IL-17F and IL-27 in the refractory group (Figure 2B). Thus, antibiotic-refractory LA was strongly associated with site-specific innate and T_H1-adaptive immune responses in joints, whereas there was a trend toward higher T_H17 responses in blood.

***Borrelia* antibody responses in Lyme disease.**

To examine the associations between inflammatory mediators and antibody responses, the levels of innate, T_H1, and T_H17 mediators were correlated with *B. burgdorferi*-specific antibodies in 29 EM patients and 79 LA

patients in whom sufficient sample volumes remained. Because no significant differences were observed in patients with refractory or responsive arthritis, these groups were combined for presentation here.

Early in the disease in EM patients, serum levels of one innate mediator (TNF), 4 T_H1 mediators (CXCL9, CXCL10, IL-12p40, CCL19), and 4 T_H17 mediators (IL-17F, IL-23, IL-25, IL-27) correlated directly with *B. burgdorferi* antibody levels (Table 1). In contrast, late in the disease in LA patients, these correlations were minimal; only one Th17 mediator, IL-17F, correlated with *Borrelia* antibodies in serum, and there was a negative correlation with IL-8 and *Borrelia* antibodies in synovial fluid. Thus, early in the infection, when large numbers of spirochetes were present and the immune response was developing, high levels of innate, T_H1, and particularly T_H17 mediators correlated with high antibody responses to the spirochete. In contrast, late in the disease when immune responses were fully matured and the levels of inflammatory mediators were generally high, these correlations were no longer observed.

Antibody responses to Lyme disease-associated autoantigens.

The levels of inflammatory mediators were then correlated with autoantibody levels to 3 Lyme disease-specific autoantigens, ECGF, apoB-100, and MMP-10, in serum of 23 EM patients or in synovial fluid of 42 LA patients in whom sufficient sample volume was still available. In serum from EM patients, ECGF autoantibodies correlated strongly with most innate mediators and with 2 T_H1 mediators, IFNγ and IL-12p70 (Table 2). Only one innate mediator (CCL2), and 3 T_H1 mediators (CXCL9, CXCL10, CCL19) correlated with MMP-10 antibodies, and there were no correlations with apoB-100 autoantibodies. Moreover, early in the infection, there were no correlations between T_H17 mediators and autoantibodies in serum.

In contrast, in LA patients, when the protein levels of these autoantigens were typically very high in synovial fluid [17-19], most T_H17 mediators, including IL-17F, IL-23, IL-25, and IL-27, correlated directly with autoantibody levels to each of the 3 autoantigens in synovial fluid (Table 2). These correlations were not as pronounced in serum (data not shown). Moreover, when LA patients were stratified into antibiotic-responsive or antibiotic-refractory groups, IL-10, a prototypical anti-inflammatory cytokine, correlated with ECGF

autoantibodies in patients with antibiotic-responsive LA. In contrast, T_H17 mediators correlated strongly with autoantibodies only in patients with antibiotic-refractory LA (Table 3), suggesting a shift to an autoimmune phenotype in these patients. Thus, although innate and T_H1-associated cytokines were often high in synovial fluid in LA patients, it was T_H17 cytokine responses that correlated strongly with autoantibodies in patients with antibiotic-refractory LA, implicating T_H17 responses in maladaptive immune processes in these patients.

DISCUSSION

In this study, T_H17 inflammatory responses correlated with *B. burgdorferi* antibody levels early in the infection in EM patients, and with autoantibody levels to self antigens late in the illness in patients with antibiotic-refractory LA. The protective or pathogenic effector functions of T_H17 cells are shaped by the composition of the local inflammatory milieu and the duration of exposure to such an environment. For example, TGFβ and IL-6 appear to be essential for early T_H17 cell lineage determination [1-4]. Such cells and their associated cytokines provide robust protection against extracellular pathogens, particularly bacteria and fungi, by rapid recruitment and activation of myeloid cells [1-3]. In contrast, prolonged or dysregulated exposure to IL-23 and IL-1β results in highly inflammatory T_H17 cells, which recruit and activate myeloid cells with capacity to cause autoimmunity and severe local tissue pathology [2]. Moreover, IL-23-induced T_H17 cells act directly on B cells and contribute to the generation of pathogenic autoantibodies that lead to onset of clinical inflammatory arthritis [36]. Studies in IL-23-deficient animals and in humans with IL-23R polymorphisms demonstrate that IL-23 is indispensible for the development of pathogenic T_H17 cells that lead to chronic tissue injury and autoimmunity [1, 2, 5-8]. In human Lyme disease, which occurs in stages, one may observe both types of T_H17 responses at different stages of the disease.

During early infection, the abundance of *B. burgdorferi* antigens in EM skin lesions leads to increased levels of innate, T_H1, and T_H17-associated inflammatory mediators which appear to help control the infection.

Additionally, the infection triggers antibody responses to *B. burgdorferi*, and in some patients, non-pathogenic autoantibodies develop [17-20], presumably due to polyclonal activation of B cells [37, 38], or to close interactions between spirochetal and host lipids or proteins [39]. The fact that the 3 autoantigens studied here do not have sequence similarity with *Borrelia* proteins speaks against the T cell epitope mimicry hypothesis [17-19]. Although some EM patients have pronounced T_H17 responses with particularly high levels of IL-23, the responses at this stage appear to be protective and correlate directly with the levels of *B. burgdorferi* antibodies but not with autoantibodies. In contrast with innate and T_H1 mediators which decline markedly during convalescence, T_H17 mediators decline more slowly, and this proclivity to remain elevated may become disadvantageous. In a prospective study of European patients with EM, high IL-23 levels sometimes persisted for many months after antibiotic therapy and were associated with post-Lyme symptoms and ECGF autoantibodies [30].

These responses appear to change in LA patients, particularly in those with refractory LA, presumably due to differences in the local inflammatory environment and a shift towards high levels of self antigens associated with immune processes in joints. Patients with antibiotic-refractory LA, have exceptionally high levels of IL-6 in synovial fluid (~15,000 pg/ml versus ~10 pg/ml in EM sera), which is essential for T_H17 lineage specification. In addition, most patients have elevated levels of IL-23, which would presumably polarize T_H17 cells towards a pathogenic phenotype over the prolonged course of the disease, leading to recruitment of inflammatory myeloid cells that can induce local tissue injury [2, 3]. Moreover, emerging evidence demonstrates that IL-23-activated T_H17 cells control the inflammatory activity of autoantibodies by regulating their glycosylation state on newly differentiating plasmablasts, thereby contributing to the shift from asymptomatic to clinical onset of autoimmune arthritis [36]. These findings demonstrate a unique linkage between T_H17 immunity and pathogenic autoantibody responses, which appears absent with other mediators such as CCL19, CXCL9, and CXCL10 that have also been implicated in dysregulated immune responses after EM [40] or LA [23-25]. Thus, in some patients, the prolonged exposure to a chronic inflammatory environment,

particularly high levels of IL-23, and the predominance of autoantigens in joints likely facilitates T_H17-associated autoimmune responses.

As evidenced by recent work in RA, a prototypic chronic inflammatory arthritity, T_H17 immunity, in the context of IL-23 stimulation, not only sets the stage for autoimmune responses, but also contributes to affinity maturation and post-translational modifications of autoantibodies, both of which are necessary for pathogenicity and clinical-evidenced autoimmune disease in joints [36]. We postulate that a similar scenario may occur in patients with Lyme disease in whom autoantibodies may be present during several stages of disease, but appear to become pathogenic primarily in the subgroup of patients with antibiotic-refractory LA. The correlation of autoantibody responses with elevated IL-10 levels in patients with antibiotic-responsive LA suggests that these patients have the ability to control autoreactive responses and their immune system regains homeostasis following the resolution of the infection. In contrast, in patients with antibiotic-refractory LA, the combination of genetic predisposition, abundance of autoantigens in joints, and prolonged exposure to high levels of T_H17 mediators such as IL-23, leads to a shift towards immunoreactive autoantibody responses with pathogenic potential, and persistent proliferative synovitis for months to years after antibiotic therapy.

Although an animal model of antibiotic-refractory LA is lacking, a unique mouse model supports a role for T_H17 immune responses in severe Lyme arthritis. C57/BL/6 mice vaccinated with formalin-inactivated *B. burgdorferi* followed 3 weeks later by infection with live spirochetes developed severe destructive arthritis [26, 28, 29]. This manipulation appears to lead to the development of pathogenic T_H17 cells, since the administration of neutralizing antibodies against IL-17 or IL-23 delayed the onset of joint swelling and ameliorated the histological changes associated with destructive arthritis. This was accompanied by a decrease in CD4+ IL-17-producing cells and an increase in CD4+CD25 T cells, presumably of a regulatory phenotype [29].

In summary, our findings demonstrate that T_H17 responses span the clinical spectrum of Lyme disease from host defense against the causative pathogen during early infection to autoimmunity late in the disease in patients with antibiotic-refractory LA. It will be important to learn whether similar dysregulated immune responses may play a role in other manifestations of Lyme disease or post-Lyme phenomena.

NOTES:

Author contribution

K.S. designed the study. K.S., K.B.S., A.P., and J.C. conducted the experiments and the data analyses for this study. A.C.S. and S.A. provided the patient samples and clinical information. K.B.S., J.C., and A.P. helped with design and interpretation of experiments involving autoantibodies. K.S., A.P., and A.C.S. wrote the manuscript. A.A. and R.S. performed the statistical analyses, contributed to generation of Tables, and helped with the interpretation of the data. All authors reviewed and approved the manuscript.

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Conflicts of interest

The authors do not have associations that may cause a conflict of interest, unless disclosed otherwise in the COI forms.

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FIGURE LEGENDS

Figure 1. Cytokine and chemokine levels in acute and convalescent serum samples from patients with erythema migrans. Protein levels of 21 mediators associated with innate, T_H1, or T_H17 immune responses were assessed in 91 culture-positive patients with erythema migrans (EM) using bead-based Luminex assays. **Panel A**, comparison of cytokine and chemokine levels in matched acute and convalescent serum samples from patients with EM or in healthy controls. Black bars represent acute serum samples obtained prior to antibiotic therapy, a period of active infection. Gray bars represent convalescent samples obtained at the conclusion of antibiotic therapy, ~ 3 weeks after study entry. White bars represent values in healthy controls. The bars represent the mean values and I-bars represent the standard error of the mean. P values for comparison of acute and convalescent samples are indicated above the bars, p values for comparison with healthy controls are indicated by an asterisk (*P≤0.05, ** P≤0.01, ***P≤0.001). **Panel B,** cytokine and chemokine values in EM patients stratified according to the presence of symptoms: patients with no associated symptoms (N=21, white box plots), 1-5 symptoms (N=29, light gray box plots) or 6-20 symptoms (N=41, dark gray box plots) at first visit. The box represents 25-75 percentile, the line inside the box represents the median value, and I-bars represent 10-90 percentile. P values for comparison of patietns with 1-5 symptoms versus 6-12 symptoms are indicated above the box plots, P values for comparison of patients with 1-5 symptoms or 6-20 symptoms to those with no symptoms are indicated by an asterisk (*P≤0.05, ** P≤0.01, ***P≤0.001).

Figure 2. Cytokine and chemokine levels in patients with Lyme arthritis. Protein levels of 21 mediators associated with innate, T_H1, or T_H17 immune responses were assessed in serum and synovial fluid from 141 patients with Lyme arthritis (LA) using bead-based Luminex assays. **Panel A**, comparison of cytokine and chemokine values in serum or synovial fluid samples from patients with LA or in healthy controls. Black bars represent synovial fluid samples, gray bars represent serum samples, and white bars represent values in healthy subjects. The bars represent the mean values and I-bars represent the standard error of the mean. P values for

comparison of synovial fluid and serum are indicated above the bars, p values for comparison with healthy controls are indicated by an asterisk (*P≤0.05, ** P≤0.01, ***P≤0.001). **Panel B,** cytokine and chemokine values in serum (upper panel) or synovial fluid (lower panel) in LA patients stratified according to antibiotic-responsive (white box plots, N=60) or antibiotic-refractory (gray box plots, N=81) course. The box represents 25-75 percentile, the line inside the box represents median values, and I-bars represent 10-90 percentile. Because of the large range in IFNγ and IL-23 levels, the highest values for these mediators may be listed in parenthesis above the graphs. P values for comparison of patients with antibiotic-responsive or refractory LA are indicated by an asterisk (*P≤0.05, ** P≤0.01).

Table 1. Correlation of inflammatory mediators with *Borrelia* IgG antibody responses in patients with erythema migrans or Lyme arthritis*

	***Borrelia* antibody responses (IgG)**		
	EM Serum (N=29)	**LA Serum** (N=79)	**LA Synovial Fluid** (N=44)
Innate responses			
CCL2	0.4	0.1	-0.1
	0.06	0.5	0.3
CCL3	0.2	<-0.1	-0.2
	0.4	0.7	0.3
IL-1β	0.1	-0.1	<-0.1
	0.5	0.3	1
IL-6	0.2	-0.1	<0.1
	0.3	0.2	1
IL-8	-0.1	<0.1	**r = -0.5**
	0.7	0.8	**p = 0.003**
IL-10	0.2	<-0.1	<0.1
	0.3	0.6	0.8
TNF	**r = 0.4**	<-0.1	-0.1
	p = 0.04	0.6	0.4
IFNα	0.3	<0.1	<0.1
	0.08	0.6	0.9
TH1 Adaptive responses			
IFNγ	0.1	<-0.1	<0.1
	0.7	0.7	1
CXCL9	**r = 0.4**	<0.1	-0.2
	p = 0.03	1	0.3
CXCL10	**r = 0.4**	<-0.1	<-0.1
	p = 0.02	0.5	0.6
IL-12p40	**r = 0.4**	-0.1	<0.1
	p = 0.03	0.4	0.9
IL-12p70	0.3	<0.1	0.2
	0.1	0.7	0.3
CCL19	**r = 0.4**	0.2	-0.2
	p = 0.04	0.07	0.2
TH17 Adaptive responses			
IL-17A	r = 0.3	<-0.1	-0.2
	p = 0.07	0.6	0.2
IL-17F	**r = 0.4**	**r = 0.3**	0.2
	p = 0.02	**p = 0.02**	0.3
IL-23	**r = 0.4**	0.2	0.2
	p = 0.02	0.2	0.4
IL-25	**r = 0.5**	<0.1	0.2
	p = 0.01	0.5	0.2
IL-27	**r = 0.4**	<0.1	0.2
	p = 0.02	0.5	0.2

*Correlations between the levels of inflammatory mediators (pg/ml) and *Borrelia* antibodies (OD450) were performed using a Pearson Correlation Test. Values in bold reflect statistically significant correlations based on $p \leq 0.05$ with Benjamini-Hochberg correction for multiple comparisons FDR ≤ 0.1

Table 2. Correlation of inflammatory mediators with IgG antibody responses to autoantigens in serum of patients with erythema migrans or in synovial fluid of patients with Lyme arthritis*

	EM serum IgG autoantibodies (N=23)			LA synovial fluid IgG autoantibodies (N=42)		
	ECGF	**MMP10**	**apoB-100**	**ECGF**	**MMP10**	**apoB-100**
Innate responses						
CCL2	<0.1	**r = 0.7**	-0.2	-0.1	-0.2	-0.2
	1	**p = 0.0002**	0.5	0.5	0.3	0.4
CCL3	**r = 0.7**	r = 0.4	<0.1	<0.1	-0.2	<-0.1
	p = 0.001	p = 0.07	0.9	0.7	0.2	1
IL-1β	**r = 0.8**	0.3	<0.1	0.1	-0.3	-0.2
	p = 0.0001	0.2	1	0.5	0.06	0.3
IL-6	**r = 0.6**	0.1	<-0.1	<-0.1	0.1	<-0.1
	p = 0.003	0.6	0.8	0.6	0.5	0.9
IL-8	0.3	<-0.1	0.1	<-0.1	<-0.1	-0.2
	0.3	0.8	0.6	0.6	0.8	0.3
IL-10	**r = 0.8**	0.2	0.1	0.3	0.1	0.1
	p = 0.00001	0.3	0.6	0.1	0.5	0.5
TNF	**r = 0.6**	0.3	<0.1	<-0.1	-0.2	-0.1
	p = 0.004	0.2	0.8	0.7	0.1	0.4
IFNα	**r = 0.5**	<0.1	<0.1	0.2	**r =0.4**	0.3
	p = 0.02	0.8	1	0.1	**p = 0.02**	0.1
T_H1 Adaptive responses						
IFNγ	**r = 0.8**	0.2	<0.1	<0.1	-0.2	-0.01
	p = 0.00001	0.3	0.7	0.9	0.3	0.5
CXCL9	<0.01	**r = 0.6**	0.2	<0.1	<-0.1	<-0.1
	1	**p = 0.002**	0.4	0.8	1	0.6
CXCL10	0.2	**r = 0.6**	0.4	-0.1	<-0.1	<0.1
	0.4	**p = 0.003**	0.1	0.4	1	0.9
IL-12p40	0.4	0.2	<-0.1	r = 0.3	-0.1	<0.1
	0.07	0.4	1	p = 0.04	0.5	0.9
IL-12p70	**r = 0.5**	<0.1	<-0.1	0.3	r = 0.3	0.2
	p = 0.02	1	1	0.1	p = 0.04	0.2
CCL19	0.1	**0.5**	0.4	0.2	<-0.1	<-0.1
	0.7	**0.01**	0.1	0.3	0.8	1
T_H17 Adaptive responses						
IL-17A	0.4	<0.1	<0.1	<0.1	0.3	0.2
	0.08	1	1	0.6	0.08	0.3
IL-17F	0.2	<0.1	<-0.1	**r = 0.8**	**r = 0.4**	**r =0.6**
	0.4	0.9	1	**p = 0.00000007**	**p = 0.01**	**p = 0.00007**
IL-23	0.3	<0.1	<-0.1	**r = 0.8**	**r = 0.4**	**r =0.6**
	0.2	0.9	0.9	**p = 0.00000003**	**p = 0.02**	**p = 0.00003**
IL-25	0.1	<0.1	<0.1	**r = 0.4**	**r = 0.4**	r =0.3
	0.8	0.9	1	**p = 0.006**	**p = 0.02**	p = 0.03
IL-27	0.2	<0.1	<-0.1	**r =0.7**	r = 0.3	**r =0.5**
	0.3	1	0.9	**p = 0.000005**	p = 0.05	**p = 0.001**

*Correlations between the levels of inflammatory mediators (pg/ml) and autoantibodies (OD450) were performed using a Pearson Correlation Test. Values in bold reflect statistically significant correlations based on $p \leq 0.05$ with Benjamini-Hochberg correction for multiple comparisons FDR ≤ 0.1

Table 3. Correlation of inflammatory mediators with autoantibodies to ECGF, MMP10, or ApoB-100 (IgG) in synovial fluid of patients with antibiotic-responsive or antibiotic-refractory Lyme arthritis*

	Antibiotic-responsive LA (N=15)			**Antibiotic-refractory LA (N=27)**		
	ECGF	**MMP10**	**apoB-100**	**ECGF**	**MMP10**	**apoB-100**
Innate responses						
IL-10	**r = 0.7**	0.5	0.3	<0.1	<-0.1	<0.1
	p = 0.005	0.1	0.3	0.6	0.8	0.8
IFNα	-0.3	0.3	<0.1	**r = 0.7**	r = 0.5	**r = 0.5**
	0.3	0.3	0.9	**p = 0.0002**	p = 0.02	**p = 0.008**
T_H17 Adaptive responses						
IL-17F	0.1	0.2	0.1	**r = 0.9**	r = 0.4	**r =0.6**
	0.7	0.5	0.7	**p = 0.00000003**	p = 0.03	**p = 0.0005**
IL-23	-0.1	0.2	<-0.1	**r = 0.9**	r = 0.4	**r =0.7**
	0.7	0.6	0.8	**p = 0.000000007**	p = 0.04	**p = 0.0002**
IL-25	-	-	-	**r = 0.5**	r = 0.4	r =0.4
	-	-	-	**p = 0.02**	p = 0.03	p = 0.07
IL-27	-0.4	-0.4	-0.5	**r =0.8**	r = 0.4	**r =0.6**
	0.2	0.2	0.08	**p = 0.000002**	p = 0.03	**p = 0.001**

*Correlations between the levels of inflammatory mediators (pg/ml) and autoantibodies (OD450) were performed in patients with antibiotic-responsive or refractory Lyme arthritis using a Pearson Correlation Test. Values in bold reflect statistically significant correlations based on $p \leq 0.05$ with Benjamini-Hochberg correction for multiple comparisons (FDR≤0.1). Only mediators with significant correlations are shown.

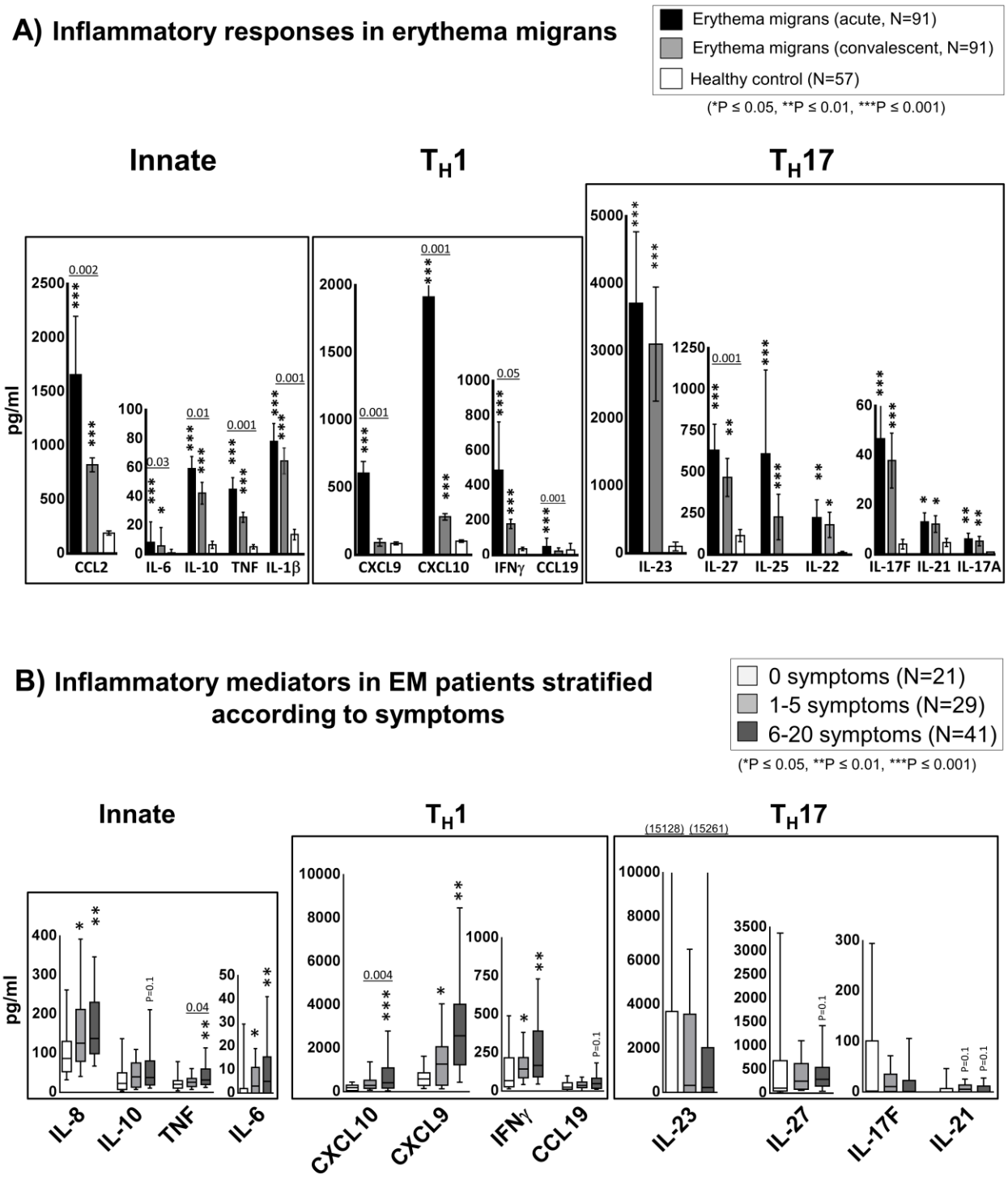

Figure 1.

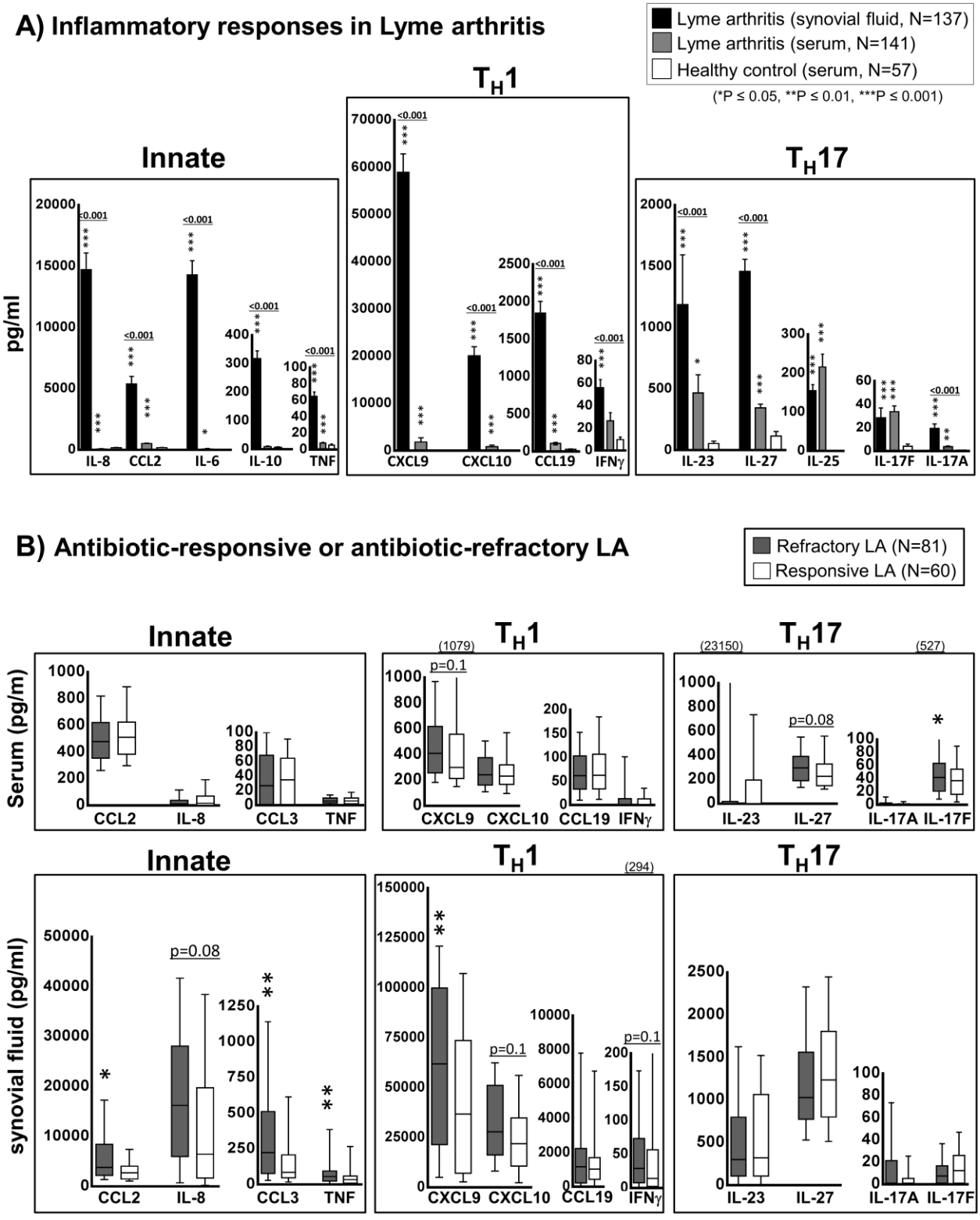

Figure 2.