OPEN

Anti-RA33 Antibodies Are Present in Patients With Lyme Disease

John B. Miller, MD, Ting Yang, PhD, Alison W. Rebman, MPH, Laura Cappelli, MD, MHS, Clifton O. Bingham, MD, Marcia Daniela Villegas de Flores, MEd, Erika Darrah, PhD, and John N. Aucott, MD

Background/Objective: To determine if anti-RA33 antibodies, which can be seen in early forms of inflammatory arthritis, are present in patients with Lyme arthritis (LA).

Methods: Anti-RA33 antibodies were tested using a commercially available assay in patients with LA (n = 47) and compared with patients with erythema migrans who returned to health (EM RTH, n = 20) and those with post-treatment Lyme disease (PTLD) (n = 50), characterized by noninflammatory arthralgia, as an observational comparative study utilizing Lyme-exposed patients from various original cohorts.

Results: We found that anti-RA33 was present in higher proportions of patients with LA (23.4% vs. 0%, $p = 0.001$) and PTLD (12.0% vs. 0%, $p = 0.040$) than healthy controls. There was also a trend toward a higher percentage of anti-RA33 positivity in patients with EM RTH versus controls (10.0% vs. 0%, $p = 0.080$). There were no statistically significant differences among groups of patients with LA, PTLD, and EM RTH ($p \geq 0.567$). There was also no difference in the proportion of patients with antibiotic-responsive LA compared with those with persistent synovitis after antibiotics, termed post-infectious LA, and there were no differences in clinical manifestations, musculoskeletal ultrasound evaluation (synovial hypertrophy, power Doppler, tendinopathy), or patient-reported outcomes based on anti-RA33 status.

Conclusions: This is the first study to identify anti-RA33 antibodies in patients with LA, though these antibodies did not identify a unique clinical subset of patients in this cohort. Unexpectedly, we found anti-RA33 antibodies at similar levels in patients with PTLD and EM RTH; further study is needed to determine the relevance of this finding.

Key Words: Lyme disease, Lyme arthritis, infectious arthritis, autoantibodies, biomarker

(*J Clin Rheumatol* 2025;31: 65–70)

From the Division of Rheumatology, Johns Hopkins University School of Medicine, Baltimore, MD.

John B. Miller, ORCID ID: 0000-0002-8438-4413

Research reported in this publication was supported in part by the Jerome L. Greene Foundation and the Rheumatology Research Foundation Career Development Funding Award: K Bridge to J.B.M. The antibody assays were performed in the NIH-funded Rheumatic Diseases Research Core Center of Johns Hopkins University (P30 AR070254). This work was supported by the Schadt Family.

E.D. is a current employee of AstraZeneca. The remaining authors declare no conflict of interest.

Correspondence: John B. Miller, MD, Division of Rheumatology, Johns Hopkins University School of Medicine, 5200 Eastern Ave, Mason F Lord Building Center Tower, Suite 4100, Baltimore, MD 21224. E-mail: JMill237@jhmi.edu.

Supplemental digital content is available for this article. Direct URL citation appears in the printed text and is provided in the HTML and PDF versions of this article on the journal's Web site (www.jclinrheum.com).

ISSN: 1076-1608
DOI: 10.1097/RHU.0000000000002176

Lyme arthritis (LA) is a painful, debilitating monoarthritis or oligoarthritis, and it is the most common late manifestation of untreated Lyme disease (LD).[1] Roughly 475,000 individuals in the United States are diagnosed with LD each year, and LA accounts for up to 30% of LD cases reported to the Centers for Disease Control and Prevention.[1,2] Up to 90% of patients with LA have clinical resolution of arthritis after appropriate antimicrobial therapy; however, despite adequate antibiotics, at least 10% of patients with LA will have ongoing inflammation of the affected joint(s), termed postinfectious LA (PILA).[3,4] This is defined by synovitis that persists at least 2 months after oral antibiotics (e.g., doxycycline, amoxicillin) or 1 month after intravenous antibiotics (e.g., ceftriaxone).[5] PILA is the immune-mediated sequela of LA, and risk is associated with specific host (e.g., HLA-DR) and *Borrelia burgdorferi* (the causative agent of LD) factors (e.g., OspA).[3,6] Immunosuppressive therapy is often required to address the pain and physical dysfunction associated with PILA.[5]

Autoantibodies have the potential to provide insight into clinical phenotypes, give useful prognostic information, and illuminate disease pathophysiology. For example, Jutras et al. hypothesized that *B. burgdorferi* peptidoglycan (PG^{Bb}) may persist in the synovium and tendons after antimicrobial therapy and contribute to the persistent inflammation, which defines PILA.[7] In this study, PG^{Bb} and anti-PG^{Bb} antibodies were only found in synovial fluid and not in serum, demonstrating the importance of a local immune response. However, the localization of antigen and anti-PG^{Bb} antibodies in the synovial fluid limits the potential clinical utility for widespread use. Steere et al. isolated HLA-DR T-cell epitopes from patients with LA and identified MMP-10, apoB-100, ECGF, and annexin A2 antibodies.[8,9] These antibodies were present in serum and were found to associate with distinct synovial tissue pathology and with risk of PILA. Other autoantibodies beyond those previously described may also have prognostic potential for LA and PILA.

Anti-RA33 targets the heterogeneous nuclear ribonucleoprotein (hnRNP) A2/B1 and is one such antibody that may have prognostic potential. These antibodies are thought to be highly specific for rheumatoid arthritis (RA), and citrullination of the RA33 antigen is hypothesized to contribute to the pathogenesis of RA.[8] However, anti-RA33 antibodies targeting the native RA33 antigen have also been described in the setting of early and undifferentiated inflammatory arthritis (IA).[9–11] Cappelli et al. found anti-RA33 antibodies in 11% (9 of 79) of patients with immune checkpoint inhibitor (ICI)–induced IA, and importantly, these antibodies were not present in patients treated with ICI therapy who did not develop IA.[12] This suggests that anti-RA33 antibodies may be a marker of immune-initiating events leading to IA.

As anti-RA33 antibodies have been detected in early, undifferentiated IA, we hypothesized that these antibodies may be present in patients with LA. No prior studies have evaluated the presence of anti-RA33 antibodies in any stage of LD.

METHODS

Patients

To determine if anti-RA33 antibodies were present in a group of patients with LA, patients with LA (n = 47) from 2 studies were included. The first cohort (n = 33) is a prospective registry following LA patients longitudinally, which allowed for characterization of a subset of LA patients who later were diagnosed with PILA (42.4%, 14 of 33). The initial study visit was used for analysis in this article, and this included patients with untreated and partially treated LA, with samples collected a median [IQR] of 13.07 months [2.62, 32.87 months] after onset of LD. In addition to collecting clinical data and biospecimens, these patients were evaluated by musculoskeletal ultrasound (MSKUS) of the affected joint(s) by one examiner (J.B.M.) using a GE Logiq e (GE, Fairfield, CT) with 12 L linear phased array transducer, with definitions for ultrasound pathology as described by OMERACT.[13] Patients completed patient-reported outcome (PRO) measures, specifically the Knee Injury and Osteoarthritis Outcome Score when the knee was affected and PROMIS (Patient-Reported Outcome Measurement Information System) including physical function (SF v1.0 10a), pain interference (SF v1.0 8a), ability to participate in social roles and activities (SF v1.0 4a), anxiety (SF v1.0 4a), depression (SF v1.0 4a), and fatigue (SF v1.0 4a). The second cohort (n = 14) included patients from a longitudinal study of patients enrolled around the time of initial LA diagnosis and antibiotic treatment. PILA status, MSKUS, and PRO measures were not available for this cohort. Samples were collected from this cohort 6 months after the end of appropriate antibiotic treatment. In both cohorts, LA was diagnosed based on the presence of a monoarthritis or oligoarthritis associated with at least 5 reactive immunoglobulin G bands on a Western blot or a positive Lyme polymerase chain reaction from the synovial fluid.

We also evaluated anti-RA33 antibodies in Lyme-exposed groups without IA, including patients with erythema migrans (EM) who returned to health after initial antibiotic treatment (RTH) (n = 20) and in patients with post-treatment LD (PTLD) (n = 50). Patients with EM RTH represent a group of patients with known LD who went on to having no lingering symptoms after the acute infection. These patients had physician-confirmed EM, and later met criteria for returning to health 6 and 12 months after appropriate antimicrobial treatment. The operational definition of return to health after LD is based on the absence of "moderate" or "severe" fatigue, pain, and/or cognitive complaints and without functional deficits at 6 and 12 months as previously described.[14] The samples used in the current analysis were collected at the time of completion of 3 weeks of antibiotic treatment. PTLD is a condition often associated with arthralgia but without synovitis or other inflammatory features on examination. Patients with PTLD, with prior medical record–confirmed and treated LD and persistent symptoms severe enough to impact function, were enrolled with samples collected on a median [IQR] of 5.17 months [4.02, 10.02 months] after onset of LD.[14]

Previously published supplemental data were also utilized to compare these cohorts with rheumatologist-confirmed RA (n = 52) from the Johns Hopkins Arthritis Center and healthy controls (n = 50) from adult volunteers with no history of autoimmune disease and no history of HIV, tuberculosis, or hepatitis infection.[12]

These investigations adhered to the Declaration of Helsinki, and the institutional review board of the Johns Hopkins University School of Medicine approved the studies from which these samples were drawn, and written informed consent was obtained from all participants prior to initiation of study-related activities.

Antibody Detection

Antibodies to the native RA33 antigen (hnRNP A2/B1) were measured in serum using a commercially available enzyme-linked immunosorbent assay kit according to the manufacturer's instructions (#ITC60015, IMETC). The cutoff value for positivity was determined using manufacturer's instructions, with a level greater than 25 units considered to be positive.

Statistical Analysis Methods

In the first analysis, we examined the differences in anti-RA33 antibody levels among 3 patient groups (LA, EM RTH, PTLD) and a healthy control group. Our primary dependent variables of interest were anti-RA33 antibody levels, treated as a continuous variable, and anti-RA33 antibody positivity, treated as a binary variable. The primary independent variable was group, whereas age and sex were considered potential confounders. Descriptive statistics summarized anti-RA33 antibody variables and confounders for all subjects by group. Comparisons between groups were conducted using Fisher exact test for categorical variables and nonparametric tests for continuous variables, accounting for small sample sizes and nonnormality of the data. Specifically, the Kruskal-Wallis test was used for overall comparisons across the 4 groups, whereas Wilcoxon rank-sum tests were used for pairwise comparisons. Group-specific boxplots and ridgeline plots were utilized to visually present the distributions and group differences. A multiple linear regression model and a multivariable logistic regression were used to assess group differences in anti-RA33 antibody variables, while adjusting for age and sex.

In the second part of our statistical analyses, we further investigated LA patients from the first cohort (n = 33) by exploring the clinical manifestations associated with anti-RA33 antibody positivity, including time from onset of joint effusion to first antibiotic, prior antibiotic treatment for LD, preceding glucocorticoid therapy, clinical serologies (rheumatoid factor [RF], anti–cyclic citrullinated peptide [CCP] antibodies, antinuclear antibodies [ANAs]), MSKUS features (synovial hypertrophy, power Doppler, tendinopathy), and PROs. Given the small sample size, Fisher exact tests were used for categorical variables, and Wilcoxon rank-sum tests were used for continuous variables.

A p value <0.05 was deemed significant. To account for the accumulation of type I errors across multiple hypothesis tests, we adjusted the p values to control the false discovery rate (FDR) at 5% for the 3 pairwise group comparisons, using the Benjamini and Yekutieli (2001) step-up FDR controlling procedure. All statistical analyses were performed using R 4.3.1.

RESULTS

We tested 117 participants for anti-RA33 antibodies, including 47 with LA; additionally, we tested Lyme-exposed patients without IA, including 20 with EM RTH and 50 with PTLD; this was compared with data from 50 previously published healthy controls and 52 individuals with RA.[12] We first studied the prevalence of anti-RA33 antibodies in LD-exposed patient groups compared with healthy controls. The Figure shows the anti-RA33 levels for these groups. Pairwise group comparisons showed that the patients with LA, PTLD, and EM RTH had significantly higher anti-RA33 antibody levels than healthy controls (values shown in the Table, pairwise comparison p values <0.001 were separately calculated). We also found that compared with healthy controls, anti-RA33 antibodies were present in higher proportions of patients with LA (23.4% vs. 0%, $p = 0.001$) and PTLD (12.0% vs. 0%, $p = 0.040$). Although not statistically significant, there was a trend toward a higher percentage of anti-RA33 positivity in EM RTH (10.0% vs. 0%, $p = 0.080$) compared with healthy controls. However, there were

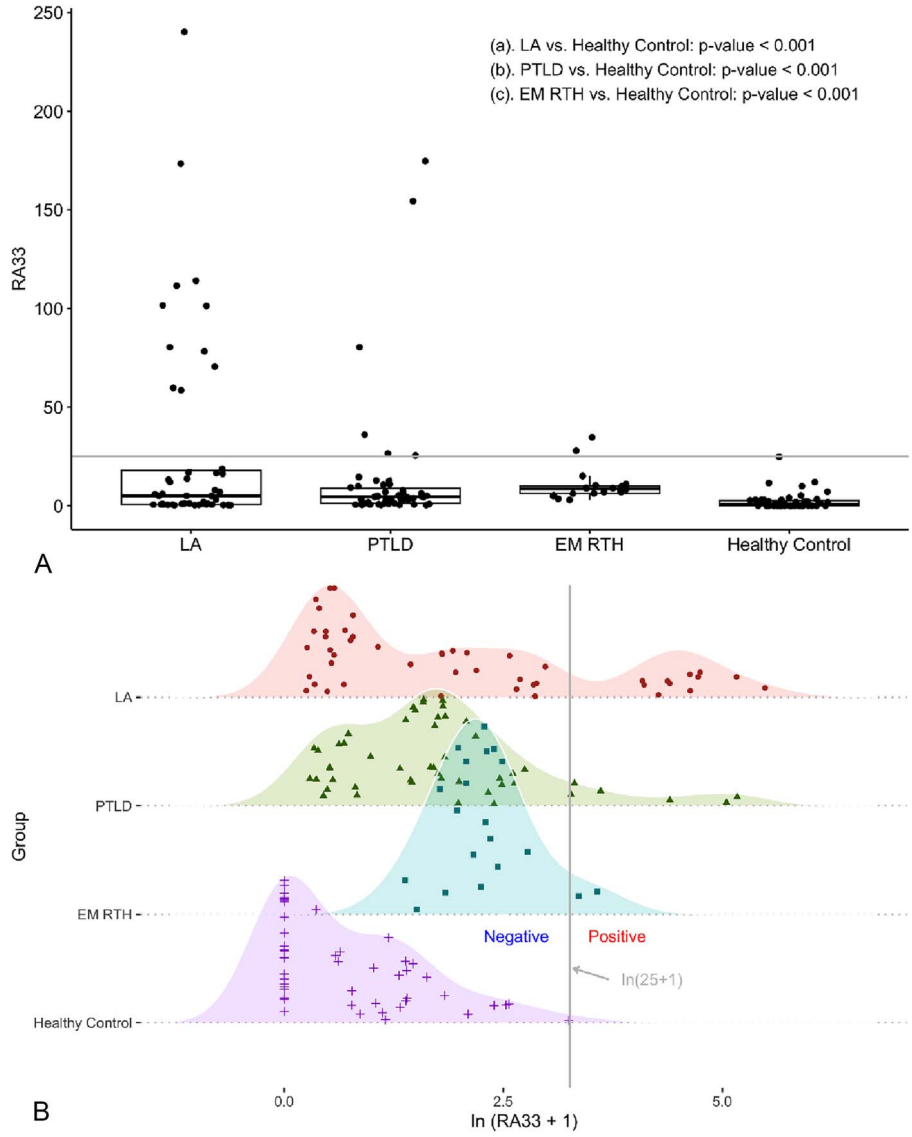

FIGURE. A, Distribution of anti-RA33 antibody levels in arthritis LA, PTLD, EM RTH, and healthy controls. B, Ridgeline plots of natural log-transformed anti-RA33 antibody level via a $\log(x + 1)$ transformation for an easier visualization of the data with skewness. 1. Dot plots were overlayed on the ridgeline plots with a small amount of random noise added to create better separation of values too close to one another; 2. *p* values are based on Wilcoxon rank-sum tests for pairwise comparisons with the healthy control group and have been adjusted to control the FDR at 5% across the 3 pairwise group comparisons, using the Benjamini and Yekutieli (2001) step-up FDR controlling procedure.

TABLE. Age, Sex, and Anti-RA33 Antibody Positivity, by Group

	LA	PTLD	EM RTH	Healthy Control[a]	*p* value
n	47	50	20	50	
Age (median [IQR]), y	59.00 [47.00, 68.50]	48.50 [33.00, 55.75]	42.00 [30.25, 56.25]	36.50 [32.00, 45.75]	<0.001
Sex = male (%)	32 (68.1)	26 (52.0)	17 (85.0)	21 (42.0)	0.003
Anti-RA33 antibody level (median [IQR])	5.03 [0.70, 17.71]	4.53 [1.26, 8.91]	8.62 [6.25, 10.07]	0.61 [0.00, 2.74]	<0.001
Anti-RA33 antibody positive (%)	11 (23.4)	6 (12.0)	2 (10.0)	0 (0.0)	0.001

Categorical variables are reported as counts with percentages. Continuous variables were reported as medians with [25th percentile, 75th percentile], considering small sample sizes and nonnormality of the group-specific data.

[a] Data previously published.

no statistically significant differences in the percentage of anti-RA33 positivity among groups of patients with LA, PTLD, and EM RTH ($p \geq 0.567$). Additionally, anti-RA33 antibodies were more prevalent in patients with LA when compared with the previously published RA cohort, using the manufacture-defined cutoff value of >25 antibody units (23.4% vs. 3.8%, $p = 0.006$).[12]

We observed significant differences in age and sex among the 4 participant groups (Table). Regression models adjusted for age and sex yielded results consistent with the unadjusted analyses (Supplemental Tables 1 and 2, http://links.lww.com/RHU/A733). Considering that LA patients came from 2 separate studies, we also examined the potential differences in anti-RA33 antibodies between the 2 study cohorts and found that the percentage of patients with anti-RA33 antibodies was not statistically significantly different between these cohorts (27.3% vs. 14.3%, $p = 0.464$).

Data from patients with LA enrolled in the first study allowed us to characterize later PILA status at subsequent study visits. Among the patients with LA in this study cohort, 42.4% (14 of 33) developed PILA, with 57.6% returning to health after completing antimicrobial therapy. We found no statistically significant difference in the median anti-RA33 antibodies in patients with PILA compared with those with antibiotic-responsive LA (9.38 [1.54, 48.02] vs. 7.04 [1.19, 48.59], $p = 0.827$), or the percentage of anti-RA33 antibody positivity (28.6% vs. 26.3%, $p = 1.000$) between the 2 groups.

Of the LA patients from the first study (n = 33), data on clinical manifestations, antibody tests, and ultrasound results were available for subsets of patients of various sample sizes. We found no statistically significant differences in these factors between LA patients who were anti-RA33 antibody positive and those without anti-RA33 antibodies (Supplemental Table 3, http://links.lww.com/RHU/A733), although this could partially be a result of small sample sizes. Specifically, we found no significant differences in time from onset of joint effusion to first antibiotic (n = 24, $p = 0.690$), time from joint effusion to anti-RA33 testing (n = 27, $p = 0.504$), antibiotic use before study enrollment (e.g., doxycycline, amoxicillin) (n = 29, $p \geq 0.799$), or preceding glucocorticoid therapy (n = 29, $p = 0.901$) between LA patients who were anti-RA33 antibody positive and negative.

Additional antibody testing, including ANAs, RF, and anti-CCP antibodies, was available for 17 patients. RF and anti-CCP were not detected in any of the LA patients. Although not statistically significant, there was a trend toward a higher prevalence of ANAs in the group with anti-RA33 antibodies (50.0% [3 of 6] vs. 18.2% [2 of 11], $p = 0.280$) with no trend toward ANA pattern. Additionally, we did not find any significant difference in ANA positivity between patients with antibiotic-responsive LA and those with PILA (33.3% [2 of 6] vs. 27.3% [3 of 11], $p = 1.000$).

Among the 19 patients with LA with available ultrasound data, we found no significant differences in synovial hypertrophy (2.0 [1.00, 2.50] vs. 2.0 [2.00, 2.25], $p = 0.557$), power Doppler (1.00 [0.00, 2.50] vs. 1.00 [0.00, 2.00], $p = 0.826$), or presence of tendinopathy (42.9% [3 of 7] vs. 50.0% [6 of 12], $p = 1.000$) between anti-RA33 antibody–positive and –negative patients at the first visit. PROMIS and Knee Injury and Osteoarthritis Outcome Score outcome measures were available for 75.8% (25 of 33) of the patients with LA from the first study. There were no statistically significant differences in either of these measures of symptom burden based on patient anti-RA33 status (Supplemental Table 4, http://links.lww.com/RHU/A733).

DISCUSSION

Anti-RA33 antibodies have never been described in the setting of LD, and our study is the first to demonstrate the presence of anti-RA33 antibodies in patients with LA. Despite the increased prevalence in LD-exposed groups compared with healthy controls, we were unable to identify unique clinical manifestations, serologies, or ultrasound results based on the presence of anti-RA33 antibodies; however, it is possible that our relatively small sample size limits the ability to detect these meaningful differences.

Anti-RA33 positivity in patients with LD is not thought to represent nonspecific B-cell activation, which can occur during infection. In our previous study evaluating autoantibodies in patients with PTLD and EM RTH, we showed that 17.2% (10 of 58) with PTLD and 20% (14 of 70) with EM RTH developed at least 1 antibody on the ANA5, Scleroderma or Myositis Euroimmun panels.[15] In that study, 21 different antibodies were found in these groups, with only 7 (anti-PMSCL75, anti-NOR90, anti-Th/To, antifibrillarin, anti–Jo-1, anti-Ro52, and anti-RP11) found in more than 1 patient with LD. Anti-Th/To was the most common antibody detected, although this was found in only 3.1% (4 of 128) of patients with LD. In contrast, our current study shows a higher prevalence of anti-RA33 antibodies in all groups with LD compared with healthy controls. Although unable to compare these data directly, our findings with RA33 antibodies, with high antibody levels in a proportion of patients with LA, are suggestive of a more specific immune response.

We unexpectedly found 12% of patients with PTLD, and 10% of patients with EM RTH also had anti-RA33 antibodies. Serum from the EM RTH cohort was collected after completion of 3 weeks of antibiotics, suggesting that these antibodies develop shortly after acute manifestations of LD. Several studies have shown that anti-RA33 antibodies are not present in healthy controls, so finding these antibodies in EM RTH, PTLD, and LA suggests that antibody development may be linked to immune-activating events that occur early during infection.

We hypothesize that anti-RA33 antibodies may develop during acute LD, a period in which up to 20% of patients develop migratory arthralgia, often with transient articular and periarticular inflammation.[1] We hypothesize that the RA33 antigen is overexpressed in the joints during this migratory joint inflammation. This has been shown in the pristane-induced arthritis model for RA, where RA33 antigen is overexpressed in the synovium prior to development of the IA, and this RA33 antigen was shown to independently increase inflammatory cytokines.[16] In this model, development of RA33 antibodies coincided with overexpression of the native antigen.

Our study found that anti-RA33 antibodies were more prevalent in patients with LA when compared with the previously published RA cohort; however, this may relate to the longer disease duration in the RA cohort. Although anti-RA33 antibodies are best characterized in RA, their true prevalence in this disease setting is unknown as there is a wide range of positivity (6%–48%) in various studies.[9,12,17,18] In part, this is thought to reflect differences of disease duration between cohorts as anti-RA33 antibodies are thought to be more prevalent in early RA and early, undifferentiated IA. In patients with IA developing within 12 months of initial evaluation, Ponikowska et al. found 37% (19 of 51) of patients with RA and 30% (7 of 23) of patients with undifferentiated IA had anti-RA33 antibodies.[11] Barbulesc et al. similarly found 48% (14 of 29) with early RA had anti-RA33 antibodies, and in this study, the patients with anti-RA33 antibodies were less likely to have synovitis by MSKUS at 1 year follow-up.[19] However, there is still debate about the relationship with disease duration as Liu et al. found no difference in the prevalence of anti-RA33 antibodies from patients with early RA compared with those with established RA.[20]

The relationship with early, undifferentiated IA is particularly interesting when we compare our data to patients with ICI-IA.[12] Cappelli et al. found that anti-RA33 antibodies were present in

13% (2 of 15) of patients with ICI-IA 1 month before ICI therapy and before development of IA, and these antibodies were not present in any of the ICI patients who did not develop IA.[12] This raises the possibility that these ICI-IA patients had either preclinical IA or other risk factors for development of IA before receiving ICI, particularly as anti-RA33 has not been shown to be present in healthy controls.[21]

Although we did not find a unique clinical phenotype based on RA33 antibodies, we are still intrigued by this antigen as a mechanism for initiation and propagation of the immune response. Overexpression and cytoplasmic localization of RA33 have been shown to occur in patients with RA and early, undifferentiated IA, with these changes occurring primarily in CD68-positive macrophages.[22] In this setting, RA33 was found to act as an autoantigen inducing T-cell responses from both synovial fluid mononuclear cells and peripheral blood mononuclear cells, resulting in increased interferon γ and interleukin 2 production.[23] These proliferative responses to the RA33 antigen were demonstrated in 60% of the patients with RA in this study, despite only 20% having antibodies to the native RA33.[23]

We propose that some of these differences may also relate to citrullination of the target RA33 antigen (citRA33).[9] In patients with RA, Konig et al.[9] showed that patients with early RA were more likely to have antibodies to the native RA33 antigen, whereas those with longstanding RA were more likely to make antibodies to citRA33. Interestingly, very few patients made antibodies to both the native and citrullinated RA33 antigen. This suggests that the native RA33 antigen may be associated with initiation of IA, whereas propagation and maintenance of the immune response rely on citrullination or other posttranslational modification. This is of particular interest in the setting of PILA, particularly as there is a link between the risk of developing PILA and shared epitope alleles (HLA-DRB1), which are also associated with increased risk of antigen citrullation.[24] Citrullination of the RA33 antigen may contribute to maintenance of the immune response and subsequent differences in phenotype between these Lyme-exposed groups.

Data from multiple studies can pose challenges due to variations in inclusion and exclusion criteria, as well as in sources of missing data. There may be other confounding factors that we were unable to assess. Other limitations include a relatively small sample size, which may limit our ability to detect clinical phenotypes associated with RA33 antibodies. Consequently, given the limitations of our sample, our study is primarily descriptive rather than causal or relational. Despite these limitations, our study adds to preexisting data that RA33 antibodies are present in early IA, although it is the first to show RA33 antibodies in an infection-associated arthritis. Development of these antibodies during acute Lyme infection potentially provides unique insights into mechanisms of arthritis initiation. Further work is needed to explore whether RA33 antibodies are linked to the migratory arthralgia associated with acute disease.

CONCLUSION

Our study is the first to demonstrate that anti-RA33 antibodies are found in a subset of patients with LA and, unexpectedly, in a subset of Lyme-exposed patients without IA, specifically patients with PTLD and EM RTH. Further work is needed to better understand the prognostic value of the anti-RA33 antibodies in these diseases.

KEY POINTS

- Anti-RA33 antibodies, which are most often associated with RA and early, undifferentiated IA, are present in a subset of patients with LA. However, these antibodies did not identify a unique clinical phenotype.
- Anti-RA33 antibodies were also unexpectedly found in patients with PTLD and patients with EM RTH after antimicrobial therapy.

REFERENCES

1. Steere AC, Schoen RT, Taylor E. The clinical evolution of Lyme arthritis. *Ann Intern Med*. 1987;107:725–731. doi:10.7326/0003-4819-107-5-725.
2. Kugeler KJ, Schwartz AM, Delorey MJ, et al. Estimating the frequency of Lyme disease diagnoses, United States, 2010–2018. *Emerg Infect Dis*. 2021;27:616–619. doi:10.3201/eid2702.202731.
3. Steere AC, Dwyer E, Winchester R. Association of chronic Lyme arthritis with HLA-DR4 and HLA-DR2 alleles. *N Engl J Med*. 1990;323:219–223. doi:10.1056/NEJM1990072632304024.
4. Miller JB, Aucott JN. Stages of Lyme arthritis. *J Clin Rheumatol*. 2021;27: e540–e546. doi:10.1097/rhu.0000000000001513.
5. Steere AC, Angelis SM. Therapy for Lyme arthritis: strategies for the treatment of antibiotic-refractory arthritis. *Arthritis Rheum*. 2006;54: 3079–3086. doi:10.1002/art.22131.
6. Lochhead RB, Strle K, Arvikar SL, et al. Lyme arthritis: linking infection, inflammation and autoimmunity. *Nat Rev Rheumatol*. 2021;17:449–461. doi:10.1038/s41584-021-00648-5.
7. Jutras BL, Lochhead RB, Kloos ZA, et al. *Borrelia burgdorferi* peptidoglycan is a persistent antigen in patients with Lyme arthritis. *Proc Natl Acad Sci U S A*. 2019;116:13498–13507. doi:10.1073/pnas.1904170116.
8. Hassfeld W, Steiner G, Hartmuth K, et al. Demonstration of a new antinuclear antibody (anti-RA33) that is highly specific for rheumatoid arthritis. *Arthritis Rheum*. 1989;32:1515–1520. doi:10.1002/anr.1780321204.
9. Konig MF, Giles JT, Nigrovic PA, et al. Antibodies to native and citrullinated RA33 (hnRNP A2/B1) challenge citrullination as the inciting principle underlying loss of tolerance in rheumatoid arthritis. *Ann Rheum Dis*. 2016;75:2022–2028. doi:10.1136/annrheumdis-2015-208529.
10. Steiner G, Smolen J. Autoantibodies in rheumatoid arthritis and their clinical significance. *Arthritis Res*. 2002;4(Suppl 2(Suppl 2)):S1–S5. doi: 10.1186/ar551 Epub 2002 Apr 26.
11. Ponikowska M, Świerkot J, Nowak B, et al. Autoantibody and metalloproteinase activity in early arthritis. *Clin Rheumatol*. 2019;38: 827–834. doi:10.1007/s10067-018-4326-5.
12. Cappelli LC, Bingham CO, Forde PM, et al. Anti-RA33 antibodies are present in a subset of patients with immune checkpoint inhibitor–induced inflammatory arthritis. *RMD Open*. 2022;8:e002511. doi:10.1136/rmdopen-2022-002511.
13. Wakefield RJ, Balint PV, Szkudlarek M, et al. Musculoskeletal ultrasound including definitions for ultrasonographic pathology. *J Rheumatol*. 2005; 32:2485–2487. doi:0315162X-32-2485 [pii].
14. Rebman AW, Bechtold KT, Yang T, et al. The clinical, symptom, and quality-of-life characterization of a well-defined group of patients with posttreatment Lyme disease syndrome. *Front Med (Lausanne)*. 2017;4:224. doi:10.3389/fmed.2017.00224.
15. Keshtkarjahromi M, Rebman AW, Antar AAR, et al. Autoantibodies in post-treatment Lyme disease and association with clinical symptoms. *Clin Exp Rheumatol*. 2024;42:1487–1490. doi:10.55563/clinexprheumatol/qcupkk.
16. Hoffmann MK, Tuncel J, Skriner K, et al. The rheumatoid arthritis–associated autoantigen hnRNP-A1 (RA33) is a major stimulator of autoimmunity in rats with pristane-induced arthritis. *J Immunol*. 2007;179: 7568–7576. doi:10.4049/jimmunol.179.11.7568.

17. Syed Mohamed Suhail SM, Nur Atiqah I, Nur Salimi Zakirah ZA, et al. Diagnostic performance of anti-RA33 antibody as a serological marker for rheumatoid arthritis. *Malays J Pathol*. 2019;41:259–265.
18. Al-Mughales JA. Immunodiagnostic significance of anti-RA33 autoantibodies in Saudi patients with rheumatoid arthritis. *J Immunol Res*. 2015;2015:1–6. doi:10.1155/2015/604305.
19. Barbulescu AL, Ciurea PL, Mitran C, et al. High frequency ultrasonography of the hand versus anti-RA33 evaluation in early rheumatoid arthritis—a pilot study. *Med Ultrason*. 2017;19:166–171. doi: 10.11152/mu-995.
20. Liu Y, Shi J, Wang B, et al. Combining calcitonin and procalcitonin and rheumatoid arthritis–related biomarkers improve diagnostic outcomes in early rheumatoid arthritis. *Dis Markers*. 2021;2021:6331994. doi:10.1155/2021/6331994.
21. Arbuckle MR, McClain MT, Rubertone MV, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med*. 2003;349:1526–1533. doi:10.1056/nejmoa021933.
22. Hayer S, Tohidast-Akrad M, Schett G, et al. Overexpression of the autoantigen hnRNP-A2 (RA33), the tumor suppressor p53 and activated MAP-kinase p38 in inflamed synovial tissue. *Arthritis Res*. 2002;4(Suppl 1):38. doi:10.1186/ar479.
23. Fritsch R, Eselböck D, Skriner K, et al. Characterization of autoreactive T cells to the autoantigens heterogeneous nuclear ribonucleoprotein A2 (RA33) and filaggrin in patients with rheumatoid arthritis. *J Immunol*. 2002;169:1068–1076. doi:10.4049/jimmunol.169.2.1068.
24. Steere AC, Klitz W, Drouin EE, et al. Antibiotic-refractory Lyme arthritis is associated with HLA-DR molecules that bind a *Borrelia burgdorferi* peptide. *J Exp Med*. 2006;203:961–971. doi:10.1084/jem.20052471.
25. Crowley JT, Strle K, Drouin EE, et al. Matrix metalloproteinase-10 is a target of T and B cell responses that correlate with synovial pathology in patients with antibiotic-refractory Lyme arthritis. *J Autoimmun*. 2016;69:24–37. doi:10.1016/j.jaut.2016.02.005.
26. Londoño D, Cadavid D, Drouin EE, et al. Antibodies to endothelial cell growth factor and obliterative microvascular lesions in the synovium of patients with antibiotic-refractory Lyme arthritis. *Arthritis Rheumatol*. 2014;66:2124–2133. doi:10.1002/art.38618.