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Evaluation of immunoreactive epitopes in the sera and cerebrospinal fluid of patients with post-treatment Lyme disease syndrome.

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ABSTRACT

While most patients fully recover after treatment for Lyme disease with recommended antibiotic regimens, some report non-specific symptoms after treatment. When these symptoms are unexplained by other conditions and persist for ≥6 months, this condition is called post-treatment Lyme disease symptoms or syndrome (PTLDS). The pathogenesis of PTLDS is unknown and no specific diagnostic biomarkers have been identified. In this study, we used a high-density peptide array to examine antibody responses to >60 primary antigens of *B. burgdorferi* from a cohort of patients diagnosed with PTLDS and recovered patients with similar Lyme disease manifestations. Using matched serum and cerebrospinal fluid (CSF), we mapped the primary reactive *B. burgdorferi* epitopes associated with PTLDS. We found that VlsE had a greater antibody response within the PTLDS cohort than recovered patients. The reactivity to OspC-specific epitopes revealed a predominance of antibodies to OspC type K and A in the PTLDS cohort. However, the major immunodominant epitopes were similar in PTLDS and recovered patients, and we were unable to identify specific diagnostic targets for PTLDS. We found a more robust reactivity in the serum over CSF and did not identify antigenic regions that were specifically associated with the infection of the central nervous system.

Keywords: post-treatment Lyme disease syndrome, Lyme disease, serology, TBD-Serochip, peptide array

INTRODUCTION

Lyme disease (LD), caused by an infection with the spirochete bacteria *Borrelia burgdorferi sensu lato (s.l.)*, is the most common tick-borne infection in the temperate regions worldwide [1,2]. In the United States, there are an estimated 476,000 people diagnosed and treated for Lyme disease yearly [3]. The infection starts at the skin at the tick bite site typically manifesting as an expanding skin lesion called erythema migrans (EM). If untreated, *B. burgdorferi* can disseminate and establish infection at distant sites. Clinically, Lyme disease is categorized as early localized, early disseminated, and late stages. Early disseminated manifestations can include multiple erythema migrans lesions, early Lyme neuroborreliosis, and Lyme carditis. Lyme arthritis is a later manifestation of the infection. [4]. Most patients fully recover after treatment with recommended antibiotic regimens. However, a proportion of patients continue to report nonspecific symptoms after antibiotic treatment. The most common complains are fatigue, difficulties with concentration and memory, joint or muscle pain, depression, anxiety, paresthesias, and sleep problems. When these symptoms are unexplained by other conditions and persist for 6 months or longer, this condition is called post-treatment Lyme disease symptoms or syndrome (PTLDS) [4,5]. The prevalence of subjective complaints 6 to 12 months after treatment ranges from 0 to 27% in prospective studies of patients with erythema migrans [6-32]. The pathogenesis of these symptoms is unknown. Most likely, several factors play a role in an individual patient, including immune dysregulation, autoimmunity, antigen or pathogen persistence, misdiagnosis, comorbidities, and psychosocial influences. [33].

Multiple approaches have been employed for analyses of clinical specimens from patients with PTLDS, but no reproducible markers have been identified [33]. Serology can provide insight into the expression and/or presence of *B. burgdorferi* antigens in PTLDS, explore autoimmunity triggered by specific antigenic fragments, and identify serologic biomarkers. In this study, we used the TBD-Serochip, a high-density peptide array to examine antibody

responses to linear epitopes from >60 primary antigens of *B. burgdorferi*. Using matched serum and cerebrospinal fluid from a cohort of PTLDS patients, comparing with recovered patients with similar LD manifestations, we identified and mapped the primary reactive *B. burgdorferi* epitopes associated with PTLDS.

METHODS

TBD-Serochip.

The **T**ick-**B**orne **D**isease Serochip (TBD-Serochip) is a slide-based peptide array used to catalogue antibody responses to tick-borne pathogens [34]. For each antigen selected for inclusion on the array, all protein sequences available as of October 2016 were downloaded from the NCBI protein database, aligned and used to design 12-mer peptides that tile each protein with an 11-aa overlap to the preceding peptide in a sliding window pattern. For *B. burgdorferi*, this included 62 different antigens (including all paralogs) that are known to elicit an antibody response in humans (**Supplemental Table 1**). For each antigen, we included the sequence of every genetic variant in the database for the 12-mer design. This included 12-mer peptides for 20 distinct outer-surface protein (Osp) C types, and a vast number of recombinant sequences for variable lipoprotein surface-exposed (VlsE). The final *B. burgdorferi* peptide component of the TBD-Serochip consisted of 91,338 peptides. The utility of the array, including its performance evaluating serum and CSF has previously been reported [34].

Cohort descriptions

Patients with Lyme disease acquired the infection in the Northeast or mid-Atlantic region of the US and fulfilled the 2017 CDC case definition of confirmed or probable Lyme disease [35]. The PTLDS cohort consisted of paired cerebrospinal fluid (CSF) and sera obtained from 41 patients (**Table 1**). We further subdivided this cohort based on the length of time from disease onset to sample collection. Group I consisted of samples from 9

patients that were collected within the first year of LD diagnosis. Group II included 23 patients with samples collected between years 1 to 5 post diagnosis, and Group III consisted of specimens from 9 patients collected 5 years or later after diagnosis. We also clustered the samples based on the primary manifestation of LD. Ten patients presented with a single EM (SEM), six had multiple EMs (MEM), ten were diagnosed with early Lyme neuroborreliosis (NB), twelve with Lyme arthritis (LA) and three presented with a flu-like illness and seroconversion. Only 2 of the 41 PTLDS patients had a history of a previous episode of Lyme disease. For both patients, the previous episode was a single erythema migrans that responded well to antibiotic therapy and both patients completely recovered. The episodes occurred 2 and 4 years before the episode that led to PTLDS symptoms. For comparative analyses, we examined 37 late convalescent-phase sera from patients with similar LD manifestations that had recovered (**Supplemental Table 2**). For CSF comparisons, we examined 8 samples from healthy individuals. In addition, we also used TBD-Serochip data from our previous study [36]. These consisted of sera from 82 patients diagnosed with LD and 85 healthy control individuals. Lyme disease patients included 27 patients with single erythema migrans, 13 patients with multiple erythema migrans, 15 patients with acute Lyme neuroborreliosis and 27 patients with Lyme arthritis. Most samples were collected after the start of antibiotic therapy. Detailed information is available in Table 3 of the previous study[36]. Samples were collected under clinical protocols approved by the National Institutes of Health (NIH) institutional review board (ClinicalTrials.gov Identifier: NCT00028080 and NCT00001539), all methods were performed in accordance with the relevant guidelines and regulations, and written informed consent was obtained from all participants.

Array data analysis

The array design and methodology have been reported previously [34,36-38]. Sera were tested at a 1:50 dilution, and CSF at 1:5. After incubation with samples and fluorescently labeled

secondary anti-IgG and anti IgM antibodies, arrays were scanned on a NimbleGen MS 200 Microarray Scanner (Roche) at 2 μm resolution, with an excitation wavelength of 532 nm for Cy3/IgM and 635 nm for Alexa Fluor/IgG, respectively. The upper limit of the signal as generated by the scanner was 65,550 RFU. Post-scanning, a file was generated that included a relative fluorescent unit (RFU) signal for each 12-mer peptide on the array. The file included 182,676 data points for *Borrelia burgdorferi*, 91338 each for IgG and IgM. Next, an aggregate file was generated by combining data files from all subarrays for each cohort. Using a set of approximately 1650 random peptides present on each subarray, we calculated the mean background signal (+3 standard deviations) and established thresholds for background signal. For serum, the mean background threshold was calculated at 10,000 RFU for IgM and 4,000 RFU for IgG. For CSF, the threshold was at 1,500 RFU for IgM and 1000 RFU for IgG. Unless specified, only peptides with a signal above the threshold on 20% of subarrays tested were retained for further analyses. For serum to CSF comparisons, we used a 1000 RFU and a 20% threshold. We considered three consecutive peptides with reactivity above background threshold as an epitope. For individual peptide analysis, we calculated the mean + 3 standard deviations for the specific peptide and set the value as the background threshold. To identify differentially reactive peptides, a variance stabilizing transformation was applied for data normalization, and a Wilcoxon Rank-Sum test was used to assess signal variation between cohorts. For time point comparisons, we used the Kruskal-Wallis test. For comparison of individual patient CSF to serum, fluorescence data without background filtration was subjected to quantile normalization for each sample and log fold changes between serum and CSF were calculated. The software and packages used included**:** R (v. 4.3.3), plotly (v. 4.10.4), dplyr (v. 1.1.4), DESeq2 (v. 1.42.1).

RESULTS

Comparison of serum to CSF in PTLDS. We first pursued a comparison of the combined serum and CSF data sets, in order to identify reactive epitopes specifically associated with central nervous system infection. After background filtration, 37,523 peptides were used for IgG comparisons. The range of immunoreactive epitopes was more expansive in the serum and we identified 1315 peptides in the serum that were less reactive or non-reactive in the CSF (**Figure 1**, **Supplemental Table 3**). Conversely, we did not identify IgG-reactive peptides that were unique to CSF. Neither serum nor CSF contained specific IgM reactivity against *Borrelia* peptides. Although some IgM-reactive peptides in sera and CSF were detected, they were mapped to fragments of FlaB that were cross-reactive in healthy controls (**Supplemental Figure 1**).

Next, we compared serum to CSF within each individual in the PTLDS group. Positive intrathecal antibody index was recorded for 13 peptides that had increased reactivity ($\leq$1.5 fold) in the CSF in $\geq$4 patients (**Table 2**). Nine of these were VlsE-associated peptides, with one peptide each from decorin binding protein (Dbp) A, OspB, OspC and OspEF-related protein (Erp).

PTLDS versus healthy controls and Lyme arthritis. We contrasted the immunoreactivity of sera from patients with PTLDS with reactivity recorded from sera of healthy controls and a cohort of 27 patients with LA that we examined previously. LA patients were seropositive by the standard two-tier criteria recommended by the US CDC, and 78% of the synovial fluids tested were positive for *B. burgdorferi* by PCR. Most samples were collected after start of antibiotic therapy [36]. The Principal Component Analysis (PCA) plots of these cohorts demonstrated that the control samples formed a distinct cluster, and the PTLDS and LA samples, while both separating from the controls, were more heterogenous and did not separate from each other (**Figure 2**). Next, we identified peptides/epitopes with highest differential levels of reactivity between PTLDS and healthy controls and generated

heatmaps for the most reactive antigens. VlsE was the most immunoreactive antigen both in terms of the number of reactive epitopes and signal intensity (**Figure 3**). Individual epitopes with the highest reactivity in PTLDS sera mapped to VlsE, FlaB, P66, BBK07, DbpA and p83/100. These also constituted the most reactive epitopes in the CSF. These were also the highest reactive epitopes in LA relative to controls (**Figure 4**). We selected a single representative 12-mer peptide with the highest reactivity within each epitope and determined its reactivity prevalence in the PTLDS and LA cohorts. The peptide within the IR6 region of VlsE had the highest reactivity and the highest prevalence, with reactivity in both PTLDS sera and CSF in 39 out of 41 patients, as well as in all the 27 LA sera (**Table 3**).

PTLDS versus Recovered. Next, we clustered the PTLDS samples into four groups, based on the main disease manifestation prior to PTLDS diagnosis (designated SEM-PTLDS (N=10), MEM-PTLDS (N=6), NB-PTLDS (N=10), and LA-PTLDS (N=12); 3 samples from patients with a flu-like illness were excluded. We then compared the reactivity of each PTLDS group to convalescent sera from recovered patients with similar LD manifestations (designated SEM-LD-REC (N=13), MEM-LD-REC (N=7) NB-LD-REC (N=9), and LA-LD-REC (N=7). VlsE was the primary antigen with greater reactivity in PTLDS, with the overall reactivity of Vls peptides elevated in SEM-PTLDS and LA-PTLDS cohorts (**Supplemental Figure 2**). When we examined reactive epitopes, we found that reactivity to peptide SGLRKVGDSVKAAS (corresponding to aa 334-347 in B31) was greater in all four PTLDS groups when compared to the recovered patients and reactivity to peptide QVADKDDPTNKFYQSV (aa 22-37) was elevated within the SEM-PTLDS group. These results are in agreement with epitopes described in Chandra et al, that reported elevated reactivity in PTLDS patients to LRKVGDSVKAASKE (aa 332-349) and SQVADKDDPTNKFYQSVIQLGNGF (aa 21-44). [39] In addition, the SEM-PTLDS cohort

had significant reactivity to DbpA and DbpB, whereas reactivity to these antigens in the SEM-LD-REC cohort was limited (**Figure 5**). Notably, the AFKDKKTGSGVSENPFIL epitope of DbpA was highly reactive in six of the ten SEM-PTLDS samples, while we recorded only one low-reactive sera to this epitope within the 13 SEM-LD-REC samples analyzed. For DbpB, the immunodominant epitope VLFEAFTGLKTGSKVTSGGLAL was reactive in five SEM-PTLDS samples, but only in one serum of the SEM-LD-REC group. However, the overall trends for other antigens indicated a lower reactivity in PTLDS when compared to non-PTLDS sera. When Vls peptides were excluded, we recorded a greater number of reactive peptides within the recovered cohorts. However, this likely reflects a longer interval between the time of illness to sample collection in the PTLDS (mean=43.6 months) compared with recovered cohort (mean=17.3 months), p=0.004

We also classified the sera of the PTLDS cohort by the length of time between diagnosis and sample collection. Comparison of the time intervals (≤1 year, 1-5 years, >5 years) did not reveal a significant variation in reactivity.

Serotyping. The design of the TBD-Serochip provides a unique opportunity to evaluate the impact of sequence diversity on antibody responses and concurrently, can enable serotyping analyses. By examining reactivity to peptides designed from variant strains of *B. burgdorferi*, we sought to determine the putative OspC type of infecting strains within the PTLDS cohort. First, we examined reactivity of LD clinical samples (N=82) to identify informative type-specific epitopes. With this approach we selected epitopes within a wide range of OspC types corresponding to aa 112-195 of B31 (accession number NP047005) (**Supplemental Figure 3**). We also selected informative fragments within the full length of VlsE (**Figure 3**), including the highly reactive fragment corresponding to B31 aa 18-33 within the N terminal region (**Supplemental Figure 4**). Applying this approach to the PTLDS cohort we putatively serotyped 33 out of 41 PTLDS sera (**Supplemental Table**

4). We found a predominance of antibodies to peptides from strains with OspC type K (N=20) and OspC type A (N=16) (**Figure 6**). Four samples had antibodies to both types. Only three other sera had signal for other serotypes, we putative reactivity to type H in two samples, and E and I in one sample each. Sera from only one of the two patients with >1 episode of Lyme disease could be serotyped. This individual only had detectable antibodies to OspC type K.

DISCUSSION

In this study we used a high-density peptide array to evaluate antibody responses to linear peptides from a cohort of patients diagnosed with PTLDS. The aim was to characterize immunoreactive fragments within the main *B. burgdorferi* antigens and potentially identify regions unique to PTLDS. Our findings demonstrated that the location of the major immunodominant epitopes were similar in patients with PTLDS and LD. We were unable to identify epitopes specific to PTLDS, or a peptide panel that could be employed as a potential diagnostic target for differentiation of PTLDS from recovered patients. Because complains of difficulties with concentration and memory are common in PTLDS, we explored the antibodies present within the CSF. We found a more robust and diverse reactivity in the serum over CSF and did not identify antigenic regions that were specifically associated with CSF. The main antibody targets were similar in both sample types, suggesting that the outer surface of *B. burgdorferi* is not significantly modified during the invasion of the central nervous system.

Only a few studies have explored the nature of antibody responses against *B. burgdorferi* in patients with PTLDS, mostly focusing on VlsE and its C6 peptide. VlsE antibody response is mostly due to IgG1 in PTLDS, a pattern similar to Lyme arthritis and convalescent samples [40]. Anti-C6 peptide antibody responses, as measured by the C6 index, showed similar decline in PTLDS and recovered patients over time [41], while another

study showed greater antibody responses to the C6 peptide and to certain VlsE epitopes in PTLDS relative to recovered patients [39]. PTLDS patients also had higher anti-*Borrelia* IgG antibodies and OspA than recovered patients [42].

We found that VlsE was the most reactive of all the antigens examined on the array and had greater overall reactivity in PTLDS patients then in the recovered cohorts. We also confirmed the location of previously reported epitopes with greater reactivity in PTLDS patients [39]. A subset of PTLDS patients had robust immunoreactivity to multiple fragments throughout VlsE. In several patients, this elevated antibody response to VlsE was present > 2 years post treatment. In addition to VlsE, we found increased immunoreactivity to peptides within DbpA and B for PTLDS patients. However, this finding was unique only to PTLDS individuals with a single EM relative to non-PTLDS single EM controls. Dbps are plasmid encoded lipoproteins essential for spirochete infectivity, whose expression is induced during transmission to vertebrate hosts. The peptide AFKDKKTGSGVSENPFIL, previously shown by us and others as a key antibody target for DbpA [34,43] was one of the overall most highly reactive epitopes in both PTLDS and LD cohorts.

As anticipated, we noted a high degree of discordance in reactivity which was based on the genotype of the infecting strains. For VlsE, samples that had numerous high reactivity to a broad range of regions for one genotype, such as B31, had limited reactivity to 297 and vice versa. These differences allowed us to attempt identify the potential infecting strain that produced the predominant serologic response. *B. burgdorferi* strains can be categorized by several genetic clusters such as RST or OspC types. The highly variable plasmid-encoded OspC lipoprotein has a critical role in infection and dissemination of *B. burgdorferi* [44-46]. Certain *ospC* genotypes of *B. burgdorferi* have a greater predilection for hematogenous dissemination while others are associated with localized skin infection [47-56]. The design of the TBD-Serochip provided a unique opportunity to evaluate the impact of sequence diversity on antibody responses and concurrently, enable serotyping

analyses. For convalescent LD sera, such analyses are more straightforward. However, because of the overall waned antibody response, serotyping the PTLDS cohort proved substantially more challenging than in our other cohorts. Nonetheless, we were able to identify a predilection for strains with OspC type K and OspC type A, in agreement with studies that have associated these genotypes with more severe disease [57,58]. However, *ospC* types K and A are also commonly found in skin of erythema migrans and ticks [58-64]. It is possible that the higher rate of anti-type K and A antibodies in PTLDS are correlated with the higher prevalence of these strains in human infection. Therefore, further studies are necessary to demonstrate enhanced virulence of these strains and have a role as a potential predisposing factor of PTLDS.

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AUTHOR CONTRIBUTIONS

ARM and RT designed the study and wrote the manuscript. AN performed the bioinformatics analyses. SSV assisted with data analysis and manuscript preparation. AE and SPN assisted with sample procurement. WIL provided intellectual input.

COMPETING INTERESTS

Dr. Marques has a patent US 8,926,989 issued; and is an unpaid Scientific Advisor to the Global Lyme Alliance and to the American Lyme Disease Foundation.

DATA AVAILABILITY STATEMENT

All data generated in this study was deposited in Dryad, accessible under the following link: http://datadryad.org/share/LINK_NOT_FOR_PUBLICATION/wihbuHkj4DfI--_sNA25PNR9SL88IR1ERwADS_RoaX0

FIGURE LEGENDS

Figure 1. Elevated IgG immunoreactivity in serum (panel A) over CSF (panel B) to DbpA, a key *B. burgdorferi* antigen. The Y axis indicates the relative amino acid coordinates of the peptides within the full-length protein from strain B31. Reactivity is shown in yellow. For clarity, the figure shows only peptides with reactivity above 10,000 RFU for serum and 2500 RFU for CSF. Sample designations are indicated on the X axis. To illustrate baseline reactivity five random control samples from each matrix were selected and shown. The asterisk shows the location of the epitope AFKDKKTGSGVSENPFIL. The 3 bars labeled 0 to 1, 1 to 5, and >5 correspond to the years from initial diagnosis to sample collection.

Figure 2. Principal Component Analysis plots comparing sera of PTLDS and healthy controls (panel A), Lyme arthritis (LA) and controls (panel B) and Lyme arthritis and PTLDS (panel C). Each point on the plot represents an individual sample.

Figure 3. IgG reactivity in the serum (panel A) and CSF (panel B) to peptides from VlsE. The maps on top show reactivity mapped to peptides from B31 strain and the bottom shows the reactivity mapped to peptides from strain 297. The Y axis indicates the relative amino acid coordinates of the peptides within the full-length protein. Reactivity is shown in yellow. For clarity, the figure shows only peptides with reactivity above 10,000 RFU for serum and 2500 RFU for CSF. Sample designations are indicated on the X axis. To illustrate baseline reactivity five random control samples from each matrix were selected. The location of the B31 epitopes INCKSQVADKDDPTNKFYQSVI and MKKDDQIAAAIALRGMAKDGKFAVKD and their corresponding sequences in 297 are indicated with * and #. The 3 bars labeled 0 to 1, 1 to 5, and >5 correspond to the years from initial diagnosis to sample collection.

Figure 4. Mean reactivity of the top 8 reactive epitopes in sera of PTLDS and LA patients. Shown is the data for a single most reactive 12-mer peptide within the epitope, indicated in red.

Figure 5. Elevated reactivity to decorin binding proteins in the SEM-PTLDS group (left) compared to SEM-LD-REC (right). Shown is the reactivity to each peptide within the immunodominant epitopes AFKDKKTGSGVSENPFIL of DbpA (top) and VLFEAFTGLKTGSKVTSGGLAL of DbpB (bottom). Signal intensity is indicated on the Y axis. The X axis lists the samples within each group.

Figure 6. Reactivity of PTLDS sera to peptides from OspC type A (left) and OspC type K (right). Shown are the peptides corresponding to aa 21-197 for OspC type A and aa 1-178 for OspC type K. The asterisks * and ** indicate the locations of the primary differential epitopes for type A and type K, respectively.

TABLES
Table 1. PTLDS cohort description

Code	Sex	Race	Age group	Time from illness to sample (years)	Main Lyme disease manifestation	Group
PTLDS_01	Male	White	20-39	> 5	NB	PTLDS
PTLDS_02	Male	White	≥60	1-5	SEM	PTLDS
PTLDS_03	Male	White	≥60	> 5	NB	PTLDS
PTLDS_04	Male	Asian	≥60	1-5	SEM	PTLDS
PTLDS_05	Male	White	50-59	1-5	LA	PTLDS
PTLDS_06	Male	White	≥60	> 5	Flu-like illness with seroconversion	PTLDS
PTLDS_07	Female	White	≥60	> 5	SEM	PTLDS
PTLDS_08	Male	White	50-59	> 5	Flu-like illness with seroconversion	PTLDS
PTLDS_09	Male	White	50-59	1-5	LA	PTLDS
PTLDS_10	Male	White	≥60	1-5	SEM	PTLDS
PTLDS_11	Female	White	40-49	1-5	MEM	PTLDS
PTLDS_12	Male	White	≥60	1-5	SEM	PTLDS
PTLDS_13	Female	White	40-49	1-5	NB	PTLDS
PTLDS_15	Male	White	≥60	1-5	LA	PTLDS
PTLDS_16	Male	White	20-39	0 to 1	LA	PTLDS
PTLDS_18	Male	White	50-59	0 to 1	LA	PTLDS
PTLDS_19	Female	White	40-49	1-5	NB	PTLDS
PTLDS_20	Female	White	50-59	0 to 1	NB	PTLDS
PTLDS_21	Female	White	50-59	> 5	LA	PTLDS
PTLDS_23	Female	White	20-39	1-5	LA	PTLDS
PTLDS_25	Male	White	50-59	0 to 1	NB	PTLDS
PTLDS_26	Male	White	50-59	1-5	LA	PTLDS
PTLDS_28	Male	White	50-59	0 to 1	MEM	PTLDS
PTLDS_29	Female	White	20-39	1-5	MEM	PTLDS
PTLDS_30	Male	White	40-49	0 to 1	MEM	PTLDS
PTLDS_31	Male	White	20-39	> 5	NB	PTLDS
PTLDS_32	Female	White	50-59	0 to 1	MEM	PTLDS
PTLDS_34	Female	White	40-49	1-5	SEM	PTLDS
PTLDS_35	Male	White	50-59	1-5	Flu-like illness with seroconversion	PTLDS
PTLDS_38	Male	White	50-59	1-5	SEM	PTLDS
PTLDS_40	Male	White	50-59	0 to 1	LA	PTLDS
PTLDS_41	Female	White	≥60	1-5	SEM	PTLDS
PTLDS_43	Male	White	40-49	> 5	LA	PTLDS
PTLDS_46	Female	White	50-59	1-5	NB	PTLDS
PTLDS_47	Female	White	50-59	1-5	LA	PTLDS
PTLDS_49	Female	White	50-59	1-5	LA	PTLDS

PTLDS_51	Male	White	50-59	1-5	SEM	PTLDS
PTLDS_52	Male	White	50-59	> 5	MEM	PTLDS
PTLDS_53	Male	White	40-49	1-5	NB	PTLDS
PTLDS_54	Male	White	50-59	1-5	SEM	PTLDS
PTLDS_56	Female	White	40-49	0 to 1	NB	PTLDS

SEM - single erythema migrans; MEM – multiple erythema migrans; NB – neuroborreliosis; LA – Lyme arthritis

Table 2. Peptides with a positive intrathecal antibody index in the CSF relative to serum within individual PTLDS patients.

Peptide	Antigen	Number of patients
KAAGAGEQDGEK	VlsE	7
GNENGAEFGQDE	VlsE	6
KGNGDGAEFDQD	VlsE	6
APKDAKQTPPAA	OspB	5
KEYGAGEDEFKE	DbpA	5
AVEDTAKAGTGG	VlsE	4
EKKEKQEVTEDN	Erp	4
GAAEQDGKKPAE	VlsE	4
GGDSASTNPDES	OspC	4
HVGKIFLKKENG	vls recombination cassette	4
KASGEDGDALKG	VlsE	4
KIFLKKENGDNH	vls recombination cassette	4
LEKAVKTAEGAS	VlsE	4

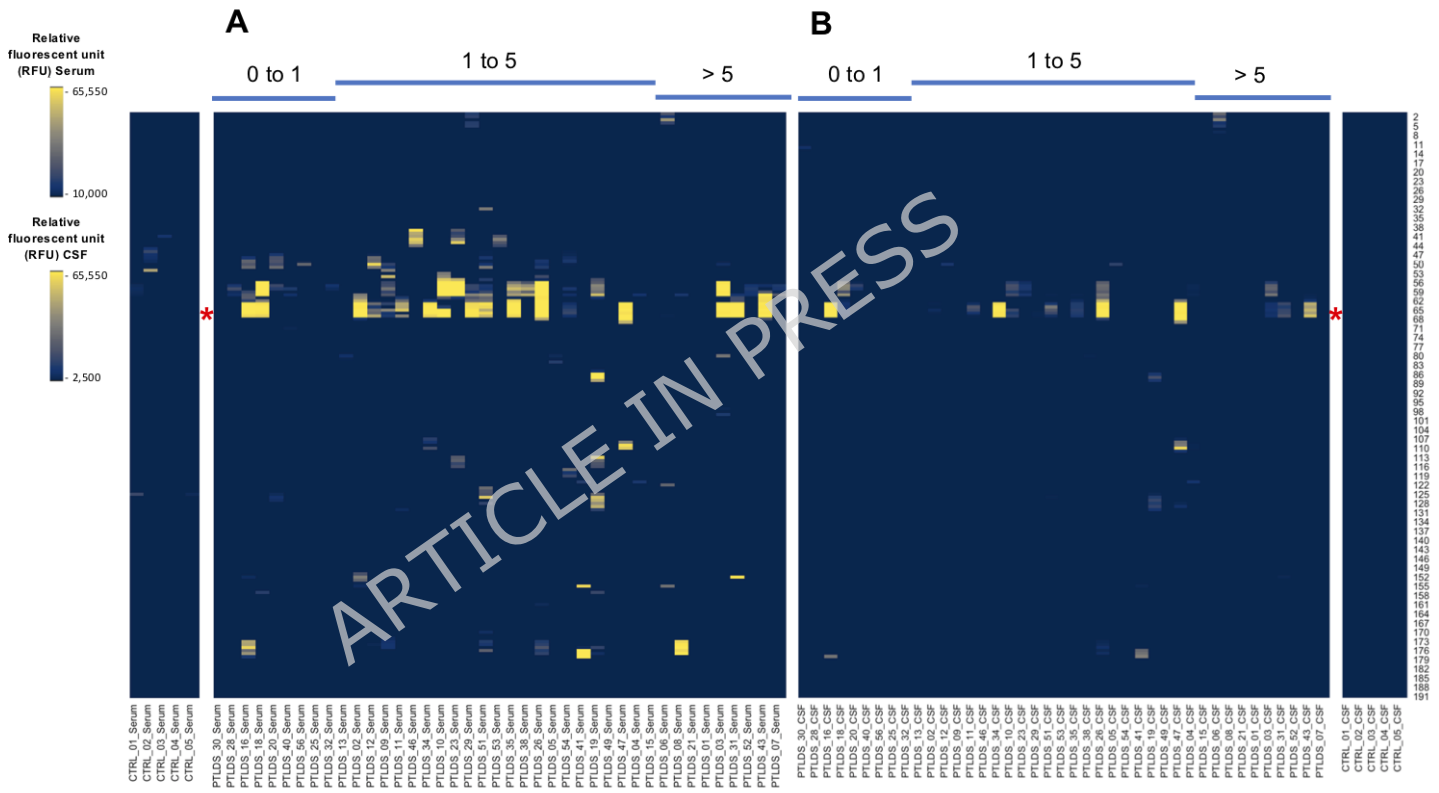
Only peptides where at least 4 of the 41 patients had >1.5-fold reactivity in the CSF versus serum are shown.

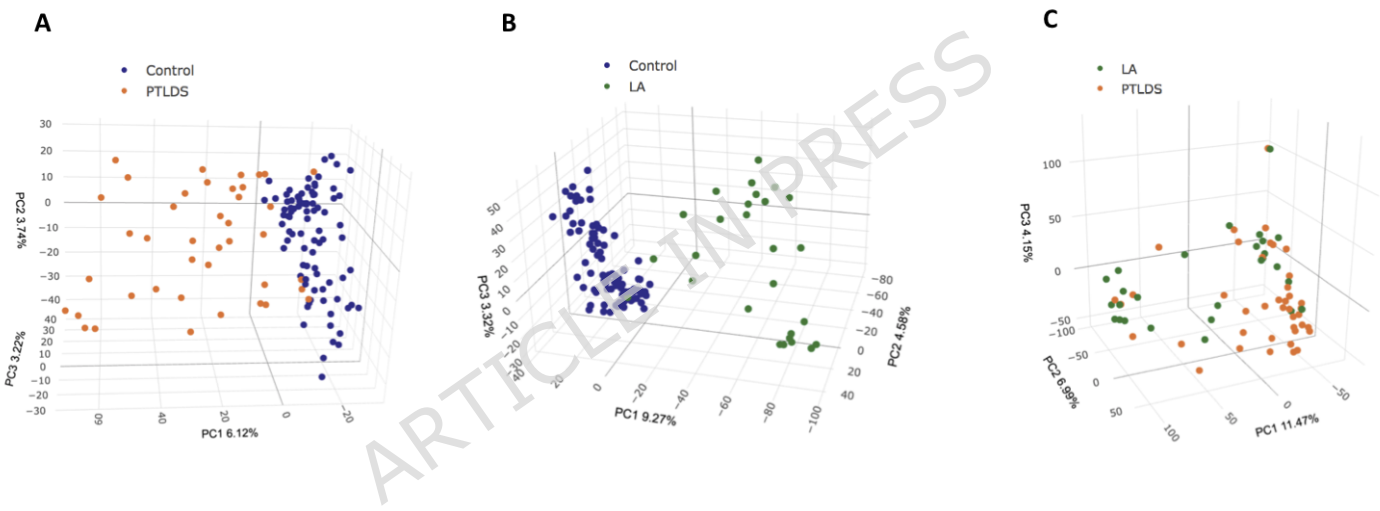
Background threshold was calculated for each peptide using mean +3 standard deviations of healthy serum controls.

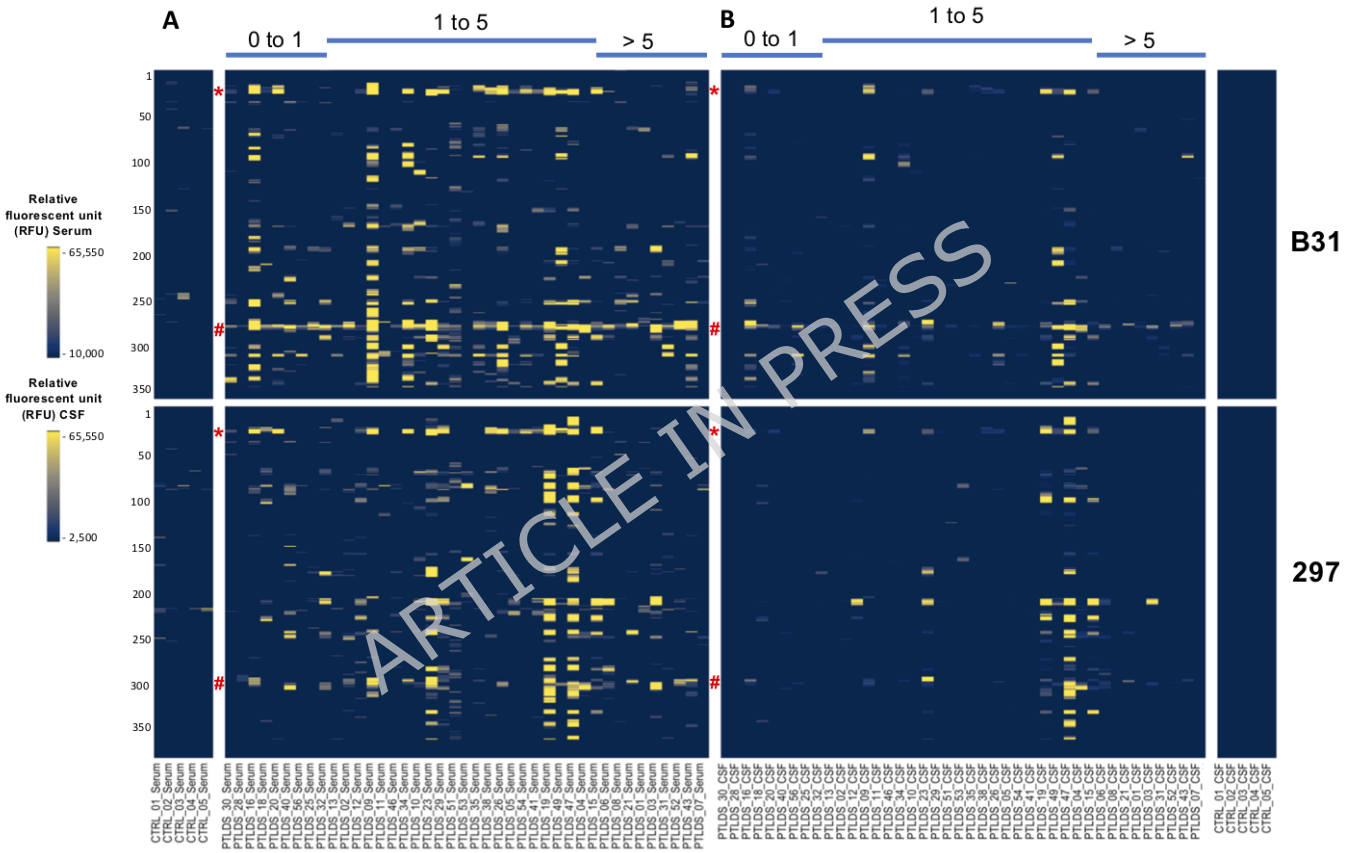
Table 3. Prevalence of the top ten reactive peptides in PTLDS and Lyme arthritis.

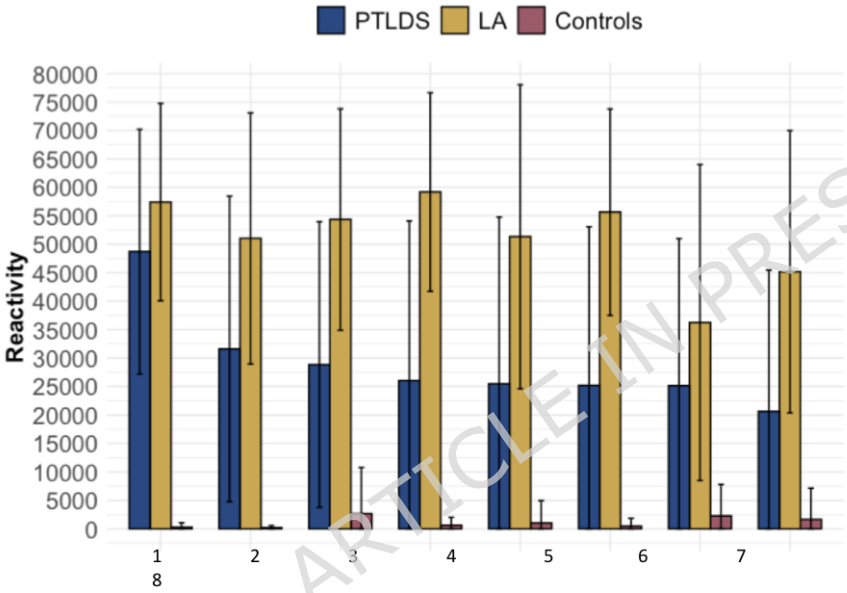
Peptide	Antigen	PTLDS CSF (N=41)	PTLDS Serum (N=41)	LA Serum (N=27)
DDQIAAAIALRG	VlsE IR6	39	39	27
QEGIQQEGAQQP	FlaB	21	30	25
ITKLTPEELENL	BBK07	25	17	23
DAGKLFAKKNDE	VlsE IR3	22	16	25
ADKDDPTNKFYQ	VlsE N-term	16	27	21
DKKTGSGVSENP	DbpA	17	17	26
NNQTEQSSTSTK	P66	16	22	16
GKALDLDRELNS	P83/100	13	14	22

Background threshold was calculated for each peptide using mean +3 standard deviations of healthy controls.









1. VlsE IR6; MKK**DDQIAAAIALRG**MAKDGKFAVKD
2. FlaB; QAAPV**QEGIQQEGAQQP**APATAP
3. BBK07; SEK**ITKLTPEELENL**AK
4. VlsE N-term; KSQV**ADKDDPTNKFYQ**SVIQ
5. DbpA; AFK**DKKTGSGVSENP**FIL
6. P66 STTE**NNQTEQSSTSTK**TTTP
7. VlsE IR4; **DAGKLFTGKNDA**
8. P83/100; SKD**GKALDLDRELNS**KASS