A Novel Human Autoantigen, Endothelial Cell Growth Factor, Is a Target of T and B Cell Responses in Patients With Lyme Disease

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Objective. Autoantigen presentation by HLA–DR molecules is thought to be a central component of many autoimmune diseases, but identifying disease-relevant autoantigens has been a difficult challenge. In this study we aimed to identify autoantigens in patients with antibiotic-refractory Lyme arthritis, in which infection-induced autoimmunity is thought to play an important role.

Methods. Using tandem mass spectrometry, naturally presented HLA–DR self peptides from a patient’s synovium were identified, synthesized, and reacted with his peripheral blood mononuclear cells (PBMCs). Immunoreactive peptides and their source proteins were then tested for T and B cell responses using large numbers of patient cells or sera.

Results. Of 120 HLA–DR–presented self peptides identified from one patient, one peptide derived from endothelial cell growth factor (ECGF) caused his PBMCs to proliferate. T and B cell responses to ECGF occurred systemically in ~10–30% of patients with early or late manifestations of Lyme disease, primarily in those with refractory arthritis–associated HLA–DR alleles, such as DRB1*0101 and 0401. Compared with patients with antibiotic-responsive arthritis, those with antibiotic-refractory arthritis had significantly higher concentrations of ECGF in synovial fluid (P < 0.0001) and more often had ECGF antibody reactivity. Among non–antibiotic-treated historical patients who developed arthritis, 26% had ECGF reactivity, which often developed before the onset of arthritis and was associated with significantly longer courses of arthritis.

Conclusion. T and B cell responses to ECGF occur in a subset of patients with Lyme disease, particularly in those with antibiotic-refractory arthritis, providing the first direct evidence of autoimmune T and B cell responses in this illness.

Presentation of autoantigens by HLA–DR molecules to CD4+ T cells is thought to be a central component of many autoimmune diseases (1). Despite the strong genetic correlation between HLA–DR alleles and autoimmunity (2), disease-relevant autoantigens presented by HLA–DR molecules have often remained elusive. Furthermore, in autoimmune diseases such as rheumatoid arthritis (RA) or lupus, multiple autoantigens are thought to be involved, and autoantibodies are often present months or years before the onset of clinical disease (3,4), suggesting that additional critical factors are required to trigger tissue pathology (3). Even so, recognition of self antigens is an essential component in the development of disease pathology.

Lyme arthritis, a late manifestation of infection...
with the tick-borne spirochete *Borrelia burgdorferi* (Bb) (5,6), provides an important human model to study questions surrounding infection-induced autoimmunity. Lyme arthritis can usually be treated successfully with 1–2 months of oral or intravenous (IV) antibiotics; the successfully treated condition is called antibiotic-responsive arthritis (7). However, in a small percentage of patients, proliferative synovitis persists for months or several years after apparent spirochetal killing with ≥3 months of oral and IV antibiotics; this is referred to as antibiotic-refractory arthritis (8).

This disease course has been postulated to result from either persistent infection, retained spirochetal antigens, or infection-induced autoimmunity (9,10). As evidence against the persistent infection hypothesis, polymerase chain reaction and culture results of synovial fluid (SF), particularly interferon-γ (IFNγ), remain high or even increase in patients with refractory arthritis during the postantibiotic period (14). In support of the autoimmunity hypothesis, specific HLA–DR alleles, particularly the DRB1*0101 or 0401 alleles, are the greatest known genetic risk factor for antibiotic-refractory arthritis (15). As in other chronic inflammatory arthropitides, HLA–DR molecules in antibiotic-refractory Lyme arthritis are intensely expressed in inflamed synovium (16).

In a search for molecular mimicry between spirochete and host proteins, partial sequence homology was found between the human peptides, LFA-1αL132–340 (17) and MAWD-BP280–288 (18), and an epitope of Bb outer surface protein A (OspA163–175) (19), which binds refractory arthritis-associated HLA–DR molecules (15). However, only a minority of patients had low-level T cell reactivity with these self peptides, and none had autoantibody responses to these self proteins (15). In a later study, Ghosh et al identified human cytokeratin 10 as a cross-reactive target ligand recognized by anti-OspA antibodies in a small group of patients with refractory arthritis (3 of 15), but not in those with responsive arthritis (0 of 5) (21). Finally, several neural proteins have been reported to induce T or B cell responses in patients with neuroborreliosis (22–24) or post-Lyme syndrome (25). However, responses against neural proteins would be unlikely to explain antibiotic-refractory arthritis.

In this study, we used discovery-based proteomics and translational research in an effort to identify autoantigens in synovial tissue, the target tissue of the immune attack in antibiotic-refractory Lyme arthritis. Based on this approach, we report here the identification of a novel autoantigen, endothelial cell growth factor (ECGF), as the target of T and B cell responses in a subset of patients with Lyme disease, thereby providing the first direct evidence of autoimmune T and B cell responses in this illness.

**PATIENTS AND METHODS**

**Patients.** All patients with Lyme disease met the Centers for Disease Control and Prevention criteria (26) and those with RA met the 2010 American College of Rheumatology/European League Against Rheumatism criteria (27). Studies conducted from 1975 to 1987 were approved by the Human Investigations Committee at the Yale University School of Medicine (New Haven, CT), those conducted from 1988 to 2002 were approved by Tufts Medical Center (Boston, MA), and those conducted after 2002 were approved by Massachusetts General Hospital.

For patients with erythema migrans (EM), who were seen from 1998 through 2001 in a study of early Lyme disease, all available peripheral blood mononuclear cell (PBMC) and serum samples from culture-positive patients were tested. For antibiotic-treated patients with Lyme arthritis, who were seen from 1988 through 2010 in a study called Immunity in Lyme Arthritis, the first available sample was used. For comparison, serum samples from non–antibiotic-treated patients who were seen in the late 1970s were tested. For patients with RA, who were evaluated from 2008 through 2010 in a study of biomarkers for early disease, PBMC, serum, and SF samples were obtained during the first year of the disease. All RA patients had positive test results for rheumatoid factor or anti–cyclic citrullinated peptide antibodies. PBMCs were stored in liquid nitrogen, and serum samples were stored at −80°C.

**Isolation and identification of synovial HLA–DR–presented peptides.** A detailed description of the isolation and identification of in vivo HLA–DR–presented peptides from patients’ synovial tissue has been published previously (28).

**T cell proliferation assay.** T cell proliferation assays were performed as previously described (29). Briefly, patients’ PBMCs were stimulated for 5 days with 2 μM of each peptide, after which 3H-thymidine was added. All nonredundant HLA–DR–presented peptides were synthesized by Mimotopes.

**Enzyme-linked immunospot (ELISpot) assay.** Assays were performed using an ELISpot kit for human IFNγ (Mabtech). ECGF peptides were synthesized and purified by high-performance liquid chromatography at the Tufts University Core Facility. The peptide sequences were as follows, with the predicted promiscuous HLA–DR binding peptides shown in boldface: A55DIRGFVAAVNGSAQGAQI; D36KVSLVL-
were standardized to a positive control included in each blot. To correct for external variation between experiments, the data of scanned blots was performed using ImageJ software. 

Nitroblue tetrazolium/BCIP substrate. Densitometric analysis

by each patient's SF sample (100 μl) and incubated overnight with plates on a platform shaker set at 200 revolutions per minute. After washing with PBS–0.05% Tween 20 (PBST), the plates were incubated with blocking buffer (5% nonfat dry milk diluted in Tris and NaCl; pH 7.5). The strips were incubated with patient serum. The sections were then incubated with anti-rabbit polyclonal ECGF (3 μg/ml) and incubated overnight at 4°C. All subsequent steps were performed at room temperature with plates on a platform shaker set at 200 revolutions per minute. After washing with PBS–0.05% Tween 20 (PBST), the plates were incubated with blocking buffer (5% nonfat dry milk in PBST), followed by patient or control serum samples (100 μl; 1:100). Subsequently, horseradish peroxidase–conjugated goat anti-human IgG was added, followed by TMB substrate. For interplate standardization, 4 patient and 8 control samples were included on each plate.

ELISA for synovial fluid ECGF. ELISA plates were coated with goat anti-human PD-ECGF (5 μg/ml) and incubated overnight at 4°C. The next day, plates were incubated with blocking buffer (5% nonfat dry milk in PBST), followed by each patient’s SF sample (100 μl; 1:100). To generate a standard curve, serial dilutions of PD-ECGF were added to each plate. The plates were then incubated with mouse anti-human PD-ECGF antibody, followed by horseradish peroxidase–conjugated goat anti-mouse IgG and then TMB substrate.

Immunohistochemistry. Synovial tissue samples were cut and fixed in cold acetone. Endogeneous peroxidases were blocked with hydrogen peroxide in methanol, followed by 1-time power block solution containing 10% normal donkey serum. The sections were then incubated with anti-rabbit polyclonal ECGF (3 μg/ml) at 4°C overnight. For negative controls, nonspecific rabbit IgG was used. The following day, the sections were incubated with biotinylated anti-rabbit secondary antibody, peroxidase–streptavidin, and then diaminobenzidine substrate. The sections were counterstained with Mayer’s hematoxylin and mounted with glycerol. Microscopic images were obtained with a Nikon Eclipse ME6000 microscope. Each slide was read under blinded conditions, and the intensity of ECGF staining on each tissue region was graded on a semi-quantitative scale of 0–3, where 0 = no ECGF-positive cells; 1 = few (∼50) positive cells; 2 = many (∼50–100) positive cells; and 3 = most cells (>100) positive.

**Statistical analysis.** Categorical data were analyzed using either the chi-square test or Fisher’s exact test, and quantitative data were analyzed by the Mann-Whitney test. All analyses were performed using SigmaStat 3.0.

**RESULTS**

Identification of HLA–DR peptides in synovial tissue. Based on the hypothesis that HLA–DR molecules in inflamed synovial tissue, the target of the immune response in antibiotic-refractory Lyme arthritis, present disease-related autoantigens, we used a broadly applicable, unbiased approach for the identification of autoantigens in this tissue. The protocol consisted of the following 3 steps: 1) a proteomics approach using tandem mass spectrometry (MS/MS) for the identification of HLA–DR–presented peptides in an individual patient’s synovial tissue, 2) synthesis and testing of all nonredundant peptides identified for T cell autoreactivity with PBMCs from the same patient, and 3) determination of whether any autoreactive peptides and their source proteins identified in a single patient also induced T and B cell responses in large numbers of patients with Lyme disease.

In step 1, we initially analyzed synovial tissue from 4 patients, 2 with antibiotic-refractory Lyme arthritis, and for comparison, 2 with RA (28). The approach is shown in Figure 1. Altogether, we identified 1,427 in vivo synovial HLA–DR–presented peptides (220–464 per patient), which were derived from 166 source proteins, including a wide range of intracellular and plasma proteins. These source proteins were substantially different from those identified from Epstein-Barr virus cell lines (30), demonstrating the necessity of using patients’ target tissues or cells for identifying naturally presented HLA–DR epitopes. Complete lists of peptides, their spectra, and their source proteins for each of the 4 patients have been published previously (28).

Screening of HLA–DR–presented peptides for autoantigenicity. In step 2, we first tested peptides identified from a patient who had a synovectomy for the treatment of antibiotic-refractory Lyme arthritis (referred to as LA1 in ref. 28). He had one of the refractory arthritis–associated HLA–DR alleles (DRB1*0101). Of the 2,237 MS/MS spectra generated from his tissue sample, 464 had a consensus match in 2 or more mass spectrometry search programs (Mascot, OMSSA, or X!Tandem), of which 104 were nonredundant. Since we wanted to test as many candidate peptides as possible, we also manually inspected the 53 peptides identi-
fied with only 1 of the 3 search programs, of which 16 could be verified. Altogether, we tested 120 nonredundant peptides for autoreactivity with the patient’s PBMCs in T cell proliferation assays. Because of limited cell numbers, peptides were pooled (3 per well) for testing.

Only 2 peptide sets (sets 33 and 40) induced proliferative responses that were >2 times background (Figure 2). When we retested the 6 peptides from these 2 sets, only 1 peptide from set 40 induced a proliferative response that was >2 times background, which was substantially higher than the response observed with any other peptide. The mass spectrum for this peptide (L340GRFERMLAAQGVDPG355) is shown in Figure 1E. This peptide was 1 of the 16 peptides identified with only 1 of the 3 search programs and originated from the source protein ECGF, also called thymidine phosphorylase or gliostatin. ECGF is a chemotactic factor, it has a proliferative effect on endothelial cells, and it induces angiogenesis (31). Moreover, it is not known to be a relevant autoantigen in any other autoimmune disease.

**Figure 1.** An overview of the isolation and identification of in vivo HLA–DR–presented peptides from patients’ synovial tissue. **A,** Antibiotic-refractory Lyme arthritis usually manifests as one swollen knee. **B,** In those cases in which therapeutic arthroscopic synovectomies are performed, 20–60 gm of inflamed synovial tissue and subcutaneous fat is removed. **C,** Immunohistologic staining of the synovial tissue shows marked exogenous expression of HLA–DR molecules. **D,** HLA–DR complexes are immunoprecipitated (IP) from synovial cell lysates. **E,** HLA–DR–presented peptides are eluted and identified by liquid chromatography tandem mass spectrometry (LC-MS/MS). The LC-MS/MS spectrum of the endothelial cell growth factor (ECGF) peptide ECGF340–355 is shown.

**Figure 2.** Screening of in vivo HLA–DR–presented peptides identified from the synovial tissue of one patient for T cell autoreactivity using the patient’s own peripheral blood mononuclear cells. All 120 nonredundant HLA–DR–presented peptides identified were synthesized and tested in sets of 3 (2 μM of each peptide). T cell proliferation was measured using a standard 3H-thymidine incorporation assay. A positive result was defined as a proliferative response >2 times background (no antigen).
period from patients with EM, the initial skin lesion of early Lyme disease, and from those with Lyme arthritis, were tested for T and B cell reactivity with ECGF. For comparison, samples from healthy control subjects and from patients with RA were tested. Although HLA–DR typing was performed only in patients with Lyme arthritis, it is likely that positivity for the HLA–DRB1*0101 and 0401 alleles would be increased among patients with RA, as in patients with antibiotic-refractory Lyme arthritis (15).

Initially, patients’ PBMCs were tested for T cell autoreactivity using commercially available recombinant ECGF. However, we found, as had others (32), that ECGF inhibited the readout of the 3H-thymidine assay, and nonspecifically induced PBMCs to secrete IFNγ Therefore, using 3 HLA–DR T cell epitope prediction algorithms (33,34), 7 T cell peptide epitopes of ECGF were identified and synthesized, including the peptide initially isolated from the patient’s synovial tissue sample (ECGF340–355). Five of the 7 peptides, including ECGF340–355, were predicted to be promiscuous HLA–DR binders (predicted to bind ≥19 HLA–DR molecules). Rather than proliferation assays, IFNγ ELISpot assays were used due to their increased sensitivity.

Healthy control subjects and RA patients had only minimal responses to a few of the 7 ECGF peptides tested (Figure 3). Of the patients with EM, an early manifestation of Lyme disease, 16% had low-level T cell responses. In contrast, 30% of the patients with antibiotic-responsive arthritis and 38% of those with antibiotic-refractory arthritis had robust responses, often to multiple ECGF peptides. Overall, patients with Lyme arthritis had substantially greater T cell responses to ECGF peptides than those in the other groups; their cells recognized all 7 peptides tested, and 10 patients had responses to 2–4 ECGF peptides, suggestive of epitope spreading.

Previously, we showed that patients with antibiotic-refractory arthritis more often had HLA–DRB1*0101, 0102, 0401, 0402, 0404, or 1501 alleles (15). In our current study, of 21 patients with refractory or responsive arthritis who had T cell reactivity with ECGF peptides, 20 (95%) had known refractory arthritis–associated alleles. Therefore, T cell responses to ECGF appear to occur primarily in patients with antibiotic-refractory risk alleles.

**Figure 3.** Testing of peripheral blood mononuclear cells (PBMCs) from healthy control subjects and patients with rheumatoid arthritis (RA), erythema migrans (EM), antibiotic-responsive Lyme arthritis, or antibiotic-refractory Lyme arthritis for T cell recognition of endothelial cell growth factor (ECGF) peptides. PBMCs were incubated with individual ECGF peptides (1 μM), phytohemagglutinin (positive control), or no peptide (negative control), and assayed by interferon-γ enzyme-linked immunospot assay. The results were quantified using a stimulation index as described in Patients and Methods, along with the sequences of the peptides. A positive response was defined as a stimulation index of ≥3 SD above the mean in healthy control subjects (the area above the shaded region). The 5 peptides predicted to be promiscuous binders were tested in all patients and control subjects. Due to limited availability of cells, the 2 peptides predicted to be nonpromiscuous binders (ECGF220–234 and ECGF302–316) were tested in only a subset of patients or control subjects (15 of the 18 healthy control subjects, none of the 12 RA patients, 18 of the 19 patients with EM, 7 of the 27 patients with antibiotic-responsive arthritis, and 11 of the 37 patients with antibiotic-refractory arthritis).
B cell responses to ECGF. ECGF-reactive CD4+ T cells likely contribute to pathogenicity by providing help to B cells to produce anti-ECGF autoantibodies. Therefore, we tested patients' serum samples for IgG anti-ECGF antibodies using 2 methods, ELISA and immunoblotting. When ECGF antibody responses were determined by ELISA, none of the 74 healthy control subjects had a positive response (defined as >3 SD above the mean value in healthy subjects) (Figure 4A).

In comparison, 15% of the patients with EM ($P = 0.001$), 8% of the patients with responsive arthritis ($P = 0.04$), and 17% of the patients with refractory arthritis ($P < 0.0001$) had positive responses. In addition, patients with antibiotic-refractory arthritis tended to have ECGF autoantibodies more frequently than those with antibiotic-responsive arthritis (17% versus 8%; $P = 0.09$). In contrast, none of the 33 patients with RA had a positive response. When these sera were tested by immunoblotting, similar results were obtained (Figure 4B), though immunoblotting was not performed in RA patients, since not enough serum remained. Moreover, the results obtained by ELISA and those obtained by immunoblotting were highly concordant in patients with refractory arthritis ($P < 0.0001$), but not in those with EM or responsive arthritis. These 2 methods may not assess all of the same epitopes, and therefore, concordance may occur only with recognition of multiple epitopes.

When concordance was assessed between T cell (ELISpot) and B cell (ELISA) assays, 13 of 22 patients (59%) with responsive arthritis and 23 of 37 patients (62%) with refractory arthritis had concordant results, and similar results were obtained when the immunoblotting data were compared. Although the overall frequencies of T and B cell immune responses in patients with EM were similar, concordance was difficult to show due to the small number of patients with positive T cell responses ($n = 3$). T or B cell reactivity to ECGF did not correlate with the duration of arthritis or how long the sample had been frozen prior to testing. Taken together, these findings indicate that T and B cell responses to ECGF occurred in patients with early or late manifestations of Lyme disease, most frequently in those with antibiotic-refractory arthritis.

Antibody responses to ECGF in patients not treated with antibiotics. In a study of the natural history of Lyme disease carried out in the late 1970s, prior to knowledge of the etiology of the illness, 55 non–antibiotic-treated patients with EM were followed up longitudinally for a median of 6 years (6). Of the 55 patients, 21 (38%) had no subsequent manifestations or only brief joint pain, whereas 34 (62%) subsequently developed intermittent attacks or persistent arthritis, lasting from 2 weeks to 4 years. Serial serum samples were still available for 42 of the 55 patients. Of the 15 patients who did not develop arthritis, 2 (13%) had ECGF antibody responses 2–3 weeks after the onset of EM, whereas 7 of the 27 patients (26%) who later had arthritis had positive ECGF antibody responses, a non-significant difference. In 6 of the 7 arthritis patients who
had ECGF responses, reactivity developed weeks to months after disease onset, prior to joint inflammation. When patients’ attacks of arthritis were added together, the duration of active arthritis was significantly longer in the 7 patients who had ECGF responses than in the 20 patients who did not (median 67 weeks versus 17 weeks, respectively; \( P < 0.004 \)). Figure 5 shows the correlation of disease activity with ECGF antibody levels in the patient who had the most prolonged arthritis. Thus, in untreated patients, ECGF antibody responses usually developed early in the illness, and in those who subsequently developed arthritis, this response was associated with a significantly longer duration of joint inflammation.

**ECGF in joint fluid and synovial tissue.** For ECGF to have pathogenic relevance as an autoantigen in antibiotic-refractory Lyme arthritis, one would predict that this protein would be present in high concentrations in patients’ inflamed SF and tissue. Although SF samples were available for patients with antibiotic-refractory arthritis, synovial tissue was not, since therapeutic synovectomies are never necessary in this patient group. As determined by sandwich ELISA, patients with antibiotic-refractory arthritis often had very high concentrations of ECGF in SF (mean 448 ng/ml) (Figure 6A), which were significantly greater than those in patients with antibiotic-responsive arthritis (mean 154 ng/ml) \( (P < 0.0001) \). RA patients also often had high levels of ECGF (mean 313 ng/ml), which is consistent

**Figure 5.** Correlation of the clinical course with IgG anti–endothelial cell growth factor (anti-ECGF) antibody responses as determined by enzyme-linked immunosorbent assay (ELISA) in a historical patient not treated with antibiotics and followed up for 6 years throughout his entire disease course. He initially had erythema migrans, followed several months later by a brief episode of arthritis, and then prolonged arthritis in a hip for 4 years. The antibody response to ECGF correlated with the 2 episodes of arthritis, and was highest during the period of prolonged arthritis. The shaded region represents the range of values found in healthy control subjects, as shown in Figure 4 (with the top of the range being 3 SD above the mean in the control subjects, and levels above this considered positive). OD = optical density.

**Figure 6.** Detection of endothelial cell growth factor (ECGF) protein in joint fluid and synovial tissue. A, ECGF concentrations in joint fluid from patients with antibiotic-responsive Lyme arthritis, antibiotic-refractory Lyme arthritis, or rheumatoid arthritis (RA) were measured by sandwich enzyme-linked immunosorbent assay. Symbols represent individual patients; horizontal lines represent the mean. B, Representative serial synovial tissue sections from a patient with antibiotic-refractory Lyme arthritis and a patient with RA stained with anti-ECGF or isotype control antibodies. Insets show higher-magnification views of the boxed areas. Circles indicate the sublining area around blood vessels; arrows indicate large cells, most likely synoviocytes.
with the findings of other investigators (32). Those investigators also showed that patients with osteoarthritis, a minimally inflammatory form of arthritis, had much lower levels (mean 8.7 ng/ml).

Synovial tissue specimens from 16 patients with antibiotic-refractory arthritis and 5 patients with RA were examined for the presence of ECGF. Of the 16 patients with antibiotic-refractory arthritis, 10 (63%) had moderate-to-intense staining for ECGF in the lining and sublining of the synovial tissue, 4 (25%) had mild staining, and 2 (12%) had no staining in these areas. In comparison, the 5 RA patients had staining for ECGF primarily in the lining area, with little seen in the sublining region. Representative examples from a patient with antibiotic-refractory arthritis and a patient with RA are shown in Figure 6B. In the synovial tissue of the patient with antibiotic-refractory arthritis, ECGF staining was clearly evident in the sublining area around blood vessels (green circles) and in large cells, most likely synoviocytes (red arrows). In contrast, in the RA patients, staining was not seen in the sublining region. Thus, the majority of patients with antibiotic-refractory arthritis had large amounts of ECGF in SF and intense staining in synovial tissue, where the protein could act as an autoantigen.

**DISCUSSION**

In this study, we used discovery-based proteomics to identify HLA–DR–presented self peptides in a patient’s synovium and determined the immunoreactivity of the peptides by testing them with his PBMCs. One peptide derived from ECGF was shown to be autoreactive. We then found that ~10–30% of patients with early or late manifestations of Lyme disease had T or B cell responses to ECGF, and they were more common in patients with antibiotic-refractory arthritis. As seen in non–antibiotic-treated historical patients, ECGF antibody responses often developed early in the illness, prior to the onset of arthritis, analogous to the situation in RA or lupus (3,4). Moreover, ECGF antibody responses in these patients were associated with more persistent arthritis. Previously, naturally presented HLA–DR peptides had been identified from other tissues and fluids by MS/MS (35–39), but until our recent study (28), not from synovial tissue. Additionally, no previous studies had systematically tested the autoreactivity of each peptide identified using PBMCs from the same patient, the key step in autoantigen identification in our present study.

ECGF itself was found in high levels in SF and synovial tissue from patients with antibiotic-refractory Lyme arthritis and those with RA. However, the protein appeared to be immunoreactive primarily in patients with Lyme disease. The reasons why Bb infection may lead to ECGF reactivity are not yet known. T cell epitope mimicry between a spirochete and host protein is often the first hypothesis considered. In a search of the Bb proteome, the Bb peptide with the greatest sequence homology with ECGF$^{340–355}$ was derived from BB_0580 (F$^{78}$ERMLA$^{83}$), a Bb protein that is not known to be immunoreactive (40). This Bb peptide has sequence homology with 6 of 9 ECGF$^{340–355}$ core residues, but in our experience (18), this is insufficient to induce cross-reactivity. Moreover, contrary to what one would expect with a molecular mimicry mechanism, no single ECGF epitope was recognized in all, or even the majority of, ECGF-reactive patients.

Alternatively, ECGF antibody responses could reflect a cross-reactive antibody response to Bb. However, the fact that patients had both T and B cell responses to ECGF, including responses to multiple T cell epitopes, is evidence against this possibility. Another option is that excessive joint inflammation may lead to bystander activation of ECGF-specific T cells. However, the lack of robust ECGF reactivity in RA patients, despite high levels of the protein in joints, is evidence against this type of nonspecific mechanism. One additional possibility is that Bb, which is known to bind several host proteins (41,42), may also bind ECGF, allowing Bb to act simultaneously as a conduit for enhanced presentation by antigen-presenting cells (APCs) and as an adjuvant.

We postulate a 3-step process in the pathogenesis of antibiotic-refractory Lyme arthritis. In the first step, autoimmune responses to ECGF develop systemically in ~10–20% of individuals with Lyme disease, often early in the illness. Both spirochete and host genetic factors are probably important in the generation of this autoimmune response. For example, Bb RST1 strains, which account for ~30–50% of the infections in the northeastern US, are more inflammatory than other Bb strains (43), and more frequently cause antibiotic-refractory arthritis (44). Important host factors likely include specific HLA genotypes, such as DRB1*0101 or 0401 (15), which are also found more often in patients who develop antibiotic-refractory arthritis.

In the second step, we propose that this rather common systemic autoimmune response becomes pathogenic in only the small percentage of patients who have marked ECGF antigen accumulation, excessive inflammation, and immune dysregulation in joints. Pa-
tients with refractory arthritis had elevated concentrations of ECGF in their SF, where it could be taken up by local APCs and presented at high concentrations to T cells, leading to their activation. Moreover, intense staining for ECGF was detected in the synovia of patients with refractory arthritis, where it could initiate immune complex deposition and tissue damage through excessive complement activation. However, more than one autoantigen or certain spirochetal antigens may be important in antibiotic-refractory arthritis, since a considerable number of patients with refractory arthritis do not have anti-ECGF antibodies. Moreover, in untreated patients, infection and autoimmunity may occur together, though the arthritis seems to be more persistent when the autoimmune component is present. It is only with antibiotic treatment that one may observe the autoimmune component independently.

Even though more than one triggering antigen may be involved, patients with antibiotic-refractory arthritis, particularly those with a TLR1 polymorphism (1805GG) (45), have exceptionally high levels of pro-inflammatory mediators, such as tumor necrosis factor α, interleukin-1β, and IFNγ, in SF and synovial tissue compared to patients with responsive arthritis (14). Although these inflammatory mediators are initially important for eradication of the spirochete, the inability to down-regulate their expression in these individuals likely contributes to immune dysregulation (46,47). Finally, in patients with antibiotic-refractory arthritis, CD4+ T effector cells in SF resist suppression by CD4+ Treg cells, and lower numbers of CD4+ Treg cells correlate with longer durations of arthritis (48).

In the third step, synovitis in most patients resolves within months to several years after antibiotic therapy, assisted by disease-modifying antirheumatic drugs such as methotrexate, which are thought to inhibit T cell activation (49). In these patients, we postulate that the innate immune “danger” signals provided by live spirochetes are no longer present, and without these signals, the adaptive immune response to autoantigens eventually regains homeostasis. Similarly, in patients requiring synovectomies, the arthritis does not usually recur because innate immune signals associated with active infection are missing.

In summary, we have shown definitively that T and B cell responses to ECGF occur in a subset of patients with Lyme disease, thereby identifying the first autoantigen that is a target of autoimmune T and B cell responses in this illness. Moreover, the approach used here for the identification of novel autoreactive HLA—DR—presented peptides in the synovia of patients with antibiotic-refractory Lyme arthritis should be valuable for the determination of immune targets in other forms of chronic inflammatory arthritis, including RA.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content and all authors approved the final version to be published. Dr. Drouin had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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REFERENCES


AUTOIMMUNITY TO ELCF IN LYME DISEASE


Clinical Images: Butterfly vertebra

The patient, a 24-year-old woman, presented with a 1-year history of mechanical low back pain without radiation to the legs. The patient’s medical history and results of the physical examination were unremarkable. A lateral radiograph of the lumbar spine showed reversal of the physiologic lordosis and reduction in the anterior height of the L4 vertebral body with a cuneiform wedging appearance (arrow in A) resembling a vertebral collapse. Two L4 hemivertebrae with a complete central cleft (arrows in B) and an abnormal, complementary L3 vertebral shape were documented by computed tomography (B) and magnetic resonance imaging (C). These radiographic findings confirmed the diagnosis of butterfly vertebra, a rare congenital malformation that results when the 2 chondrification centers in an embryonic vertebral body do not fuse. This anomaly is also known as sagittal cleft vertebra, anterior rachischisis, somatoschisis, or anterior spina bifida. It occurs most commonly in the lumbar spine, and it may be associated with complex congenital syndromes, such as Pfeiffer syndrome, Jarcho-Levin syndrome, Crouzon syndrome, and Alagille syndrome. Butterfly vertebra is generally asymptomatic and incidentally detected. Hypoplasia of one or both halves of the vertebral body can lead to lateral displacement or anterior wedging, resulting in kyphosis or scoliosis, and can be a cause of chronic back pain. Although congenital vertebral anomalies are relatively common (global prevalence 0.5–1/1,000 live births), very few cases of symmetric fusion defects resulting in butterfly vertebra have been reported. Awareness of this congenital malformation is important, since it may easily be confused with a vertebral fracture.

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