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A Novel Human Autoantigen Induces T and B Cell Responses in Patients with Antibiotic-Refractory Lyme Arthritis

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Background. Antibiotic-refractory Lyme arthritis (ARLA), defined as persistent synovitis lasting months to years after spirochetal killing with 2-3 months of oral and IV antibiotic therapy, is hypothesized to result from *B. burgdorferi*-induced autoimmunity in affected joints. When we undertook our present studies, previous attempts to identify autoantigens based on sequence homology with borrelial proteins, proteome microarrays or recombinant antibody probes had been unrevealing. Therefore, we devised an innovative new approach that combines discovery-based proteomics with translational research for the identification of naturally processed, immunogenic HLA-DR-presented autoantigens in synovial tissue.

Methods. Naturally presented HLA-DR peptides were purified from the synovial tissue of individual patients and identified by tandem mass spectrometry. Identified peptides were synthesized and tested for autoreactivity using the same patient's PBMC (MCP ref). Peptides shown to have autoreactivity were further evaluated for disease-associated T and B cell autoreactivity using PBMC and sera from a large cohort of patients with antibiotic-refractory Lyme arthritis and other comparison groups.

Results. Of the 120 non-redundant peptides identified from the synovial tissue of a single patient, only one peptide, derived from endothelial cell growth factor (ECGF), was recognized by his PBMC. Testing of PBMC from 44 additional patients with ARLA showed that 21 (48%) also had T cell autoreactivity with 1 or more of 7 T cell epitopes of ECGF, compared with 8 of 29 patients (28%) with antibiotic-responsive arthritis, 3 of 19 (16%) with erythema migrans (EM, an early disease manifestation), and 1 of 18 healthy control subjects (6%), as demonstrated by ELISPOT assay. In addition, as determined by ELISA and Western blotting, 49 of 112 patients (44%) with ARLA had IgG anti-ECGF autoantibodies with titers as high as 1:11,000, and most patients with T cell responses also had B cell reactivity with this autoantigen. In comparison with ARLA patients, antibody responses to ECGF were found in 11 of 32 patients (34%) with antibiotic-responsive arthritis (P=NS), 2 of 33 (6%) with EM (P<0.001), and 11 of 91 healthy subjects (12%) (P<0.001). ARLA patients with anti-ECGF-antibodies more often had HLA-DRB1*04 or 1501 alleles (alleles previously associated with chronic Lyme arthritis) compared with patients who lacked such autoantibodies. Finally, ECGF was found to be abundant in both synovial fluid and synovial tissue in ARLA patients, demonstrating the presence of the autoantigen at the site of inflammation.

Conclusion. About half of the study patients with ARLA had T and B cell reactivity with the human protein ECGF. This is the first autoantigen identified that induces both T and B cell autoreactivity in ARLA patients. Clinical testing for anti-ECGF autoantibodies is a promising biomarker for diagnosis, and is likely to help in treatment decisions, including the use of DMARDs after antibiotic therapy. Furthermore, this innovative, cutting-edge methodology for identifying novel autoantigens is directly applicable to any of the chronic inflammatory arthritides, including rheumatoid arthritis.