

Identification of HLA-DR-Presented Peptides in Synovial Tissue and Fluid, and PBMCs from Patients with Rheumatoid Arthritis or Antibiotic-Refractory Lyme Arthritis

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Introduction

The identification of disease-relevant autoantigens is critical to our understanding of autoimmune diseases. Therefore, we developed an unbiased, discovery-based proteomics approach for the identification of novel autoantigens and foreign antigens. Synovial tissue (the tissue under immune attack) from patients with either rheumatoid arthritis (RA) or antibiotic-refractory Lyme arthritis (ARLA) was used to extract *in vivo* HLA-DR-presented peptides (T cell antigens) and tested for autoreactivity using the patients' own cells. With this approach, ECGF was identified as the first autoantigen associated with antibiotic-refractory Lyme arthritis¹. We have expanded our protocol to include synovial fluid and peripheral blood mononuclear cells allowing us to study greater number of patients and compare the *in vivo* T cell antigen repertoire between these three compartments.

Methods

Immunoaffinity purification of HLA-DR presented peptides was performed from synovial tissue, synovial fluid, or PBMC using a previously published procedure². LC-MS/MS was performed on a nanoACQUITY UPLC (Waters Corp) coupled with an LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific). The eluent from affinity purification was injected into a reversed-phase chromatographic column and CID was used for fragmentation of selected precursor ions. Database searches were conducted with Mascot, OMSSA, and X!Tandem against the Swiss-Prot human database concatenated with a randomized decoy database. The mass tolerance was 0.01 Da for precursor ions and 0.5 Da for fragment ions. The score cutoffs for individual search programs were: Mascot ion score ? 20, OMSSA e-value ? 0.01, and X!Tandem e-value ? 10.

Preliminary Results/Abstract

HLA-DR presented peptides were purified from 5 RA and 5 ARLA patients: synovial tissue (N=9); synovial fluid (N=4); and PBMC (N=3). In one patient each with RA or ARLA, all 3 sample types were analyzed allowing for a direct comparison the HLA-DR repertoire from these 3 compartments. Data acquired from 2-to-5 LC-MS/MS runs were combined into one MGF file for each sample and the results were searched against the human protein database using 3 search engines: Mascot, OMSSA, and X!Tandem. HLA-DR-presented peptides were identified when at least 2 of the 3 protein database search programs assigned identical sequences for a given tandem mass spectrum (consensus peptides).

Altogether, 33,973 spectrum-to-peptide matches were made from the MS/MS spectra for the 16 samples. The false discovery rate of the consensus peptides for the samples was 1%. The unique consensus peptides were submitted to software generated in-house that can collate and remove redundant peptides that have amino acid sequences overlapping in a core sequence. In each sample, 57-222 non-redundant peptides were identified from synovial tissue; 2-67 from synovial fluid, and 7-17 from PBMC. Importantly, the post-translational modification of citrullination was identified in several peptides from synovial tissue and fluid from 2 RA patients, a modification strongly associated with autoreactivity in RA. However, for the most part, there was only limited overlap between the peptides identified in the 3 different compartments. The immunoreactivities of all the identified non-redundant peptides have been synthesized and are being tested against the matching patients' PBMC. Finally, the immunoreactive peptides and their source proteins are being tested for T and B cell reactivity in large numbers of patients and control subjects.

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Novel Aspect

The combined use of discovery-based MS proteomics and translational research to identify novel disease-relevant foreign and autoantigens.