using antibodies against the protein tyrosine phosphatase, CD45RO, and the chemokine receptor, CCR7. Tetramer tagged cells were magnetically enriched and analysed by flow cytometry.

Results We found a distinct population of Hcgp39-specific T cells in an RA patient at a frequency of 6/1,000,000 CD4 T cells. Interestingly, Hcgp39-specific T cells can also be detected in healthy individuals. In one blood donor, 16/1,000,000 CD4 T cells are Hcgp39 tetramers positive. Initial immunophenotyping of these self-antigen specific T cells indicates that almost the entire population is antigen-experienced in the RA patient (92%). Unexpectedly, the majority of Hcgp39-specific population detected in the healthy person also has a memory phenotype (63%), suggesting prior antigen exposure and T cell activation.

Conclusion Rare antigen-experienced Hcgp39-specific CD4 T cells can be detected in the peripheral blood of RA patients and healthy individuals. Study is currently ongoing to evaluate functional differences between these autoreactive T cell populations and to define additional self-epitopes recognised by CD4 T cells in healthy and disease states.

A190 PROFILING THE T CELL REPERTOIRE IN RHEUMATOID ARTHRITIS

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Purpose Rheumatoid arthritis (RA) is one of the most common debilitating systemic inflammatory conditions. To date, little is known about the autoantigens involved in RA and how T cells recognising self-proteins may become pathogenic in disease. The aim of this study is to determine whether autoreactive T cells in RA patients are different from those that can be found in healthy individuals.

Method Autoantigen-specific T cells are identified directly ex vivo using peptide-MHC tetramers. HLA-DR4 monomers with a tethered thrombin cleavable CLIP peptide were purified from the culture supernatant of a stable HLA-DR4 transfectant cell line by antibody affinity chromatography. The purified monomers were then biotinylated, thrombin cleaved, loaded with peptides, and oligomerised onto a streptavidin backbone labeled with a fluorophore. To identify individuals carrying the HLA-DRB1*0401 (DR4) allele, HLA typing was performed on healthy blood donors and RA patients using sequence-specific primer PCR. Individuals with DR4 allele(s) were selected for T cell repertoire analysis. To identify antigen-specific T cells, blood from DR4 positive individuals were enriched for CD4 cells by rosettasep/ficoll centrifugation. Tetramer staining was performed at room temperature for one hour using 10nM of tetramers loaded with peptides from hemagglutinin (HA) and the human glycoprotein 39 (Hcgp39). Memory phenotyping was performed