EFIS-EULAR - origin of autoantibodies

OP0325

EFFECT OF CITRULLINATION ON THE PROCESSING AND PRESENTATION OF RHEUMATOID ARTHRITIS AUTOANTIGENS

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Background: Citrullinated proteins are hallmark targets of the autoimmune response in rheumatoid arthritis (RA),¹ but the mechanism by which immune tolerance is broken to these self-proteins is poorly understood. CD4⁺ T cells are implicated as important drivers of the autoimmune response due to the high-affinity, class-switched nature of anti-citrullinated protein antibodies (ACPAs) present in the majority of RA patients and the prominent genetic contribution of certain HLA-DR alleles to RA susceptibility.^{2,3} However, the precise effect of citrullination on MHC class II antigen processing and presentation of autoantigens to CD4⁺ T cells remains unknown.

Objectives: Here we aimed to examine the hypothesis that citrullination impacts the processing and presentation of RA autoantigens via destabilization of protein folding and modification of protease cleavage sites, altering the peptide repertoire presented by antigen-presenting cells (APCs).

Methods: Using fibrinogen as a model RA autoantigen, the native and citrullinated forms were digested *in vitro* by a cocktail of lysosomal cathepsins (cathepsins B, S, and H) for proteolytic mapping, or incubated with monocyte-derived dendritic cells (mo-DCs) in a natural antigen processing assay (NAPA). Peptides generated by digestion with the cathepsin cocktail or presented by HLA-DR molecules on mo-DCs were then isolated and identified by mass spectrometry.

Results: We found that the repertoire of peptides generated by each method was altered by citrullination. By proteolytic mapping, we detected both changes in the pattern of cathepsin cleavage and an increased number of peptides in the citrullinated samples. Utilizing NAPA, we observed the creation of newly presented peptides in the citrullinated samples in some cases, and loss of presented peptides in others (Fig. 1). Strikingly, all peptides whose presentation was destroyed by citrullination contained a citrullination site. Together these results suggest that both protease cleavage and selection of peptides by HLA-DR are impacted by citrullination.

Conclusion: Citrullination alters the peptide repertoire presented by APCs. Interestingly, no citrullinated peptides were identified by NAPA, suggesting that presentation of citrulline-containing peptides to T cells may not be the primary mechanism by which tolerance is broken to citrullinated antigens. Rather, citrullination-induced destabilization of protein folding and modification of protease cleavage sites, leading to the generation of a new peptide repertoire, could play a role in activating autoreactive T cells. This mechanism could thus drive the loss of immune tolerance to the citrullinated forms of RA autoantigens.

Figure 1: Top – Distribution of fracture sites and co-existing fractures (7 most common sites shown). **Bottom** – Dendrogram of cluster analysis of 11003 patients with one or more fragility fracture. Optimal number of clusters was calculated using "Elbow method" and "Silhouette method". Clusters are defined by colour (left-right: red, green, blue) and grey dashed boxes.

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ACPA-INDUCED PAIN-BEHAVIOR, BONE LOSS AND TENDON INFLAMMATION IN MICE: A NOVEL MODEL FOR THE PRE-DISEASE PHASES OF ACPA-POSITIVE RHEUMATOID ARTHRITIS

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Background: In rheumatoid arthritis (RA), anti-citrullinated protein antibodies (ACPAs) are associated with bone loss and pain. Recently, tenosynovitis has been suggested as a predicting factor for arthritis progression in individuals at-risk for RA.

Objectives: We aimed to investigate if transfer of human ACPAs into mice could induce tenosynovitis and/or subclinical inflammation.

Methods: Monoclonal ACPA (1325:04C03 and 1325:01B09) and control (1362:01E02) antibodies (mAbs) were generated from synovial plasma or memory B cells of RA patients. 2mg of combination of monoclonal ACPAs or control antibody were injected in BALB/c female mice (age 12-16 weeks) (n= 9). Pain-like behavior was monitored by measuring mechanical hypersensitivity using von Frey filaments every 3 days and estimation by up-down Dixon method. Bone morphometrics was analyzed by micro-CT. Using specially designed mobilization casts, dedicated mouse MRI coils, and gadolinium enhanced contrast medium, the hind limbs of these mice were scanned in a 9.4 T scanner and resulting T1-weighted images were evaluated for signs of soft tissue joint inflammation. The MRI images were scored for the presence of joint involvement and tendon inflammatory changes by 3 readers in a blinded manner.

Results: ACPAs (1325:04C03 and 1325:01B09) induced pain-like behavior (lasting for at least 4 weeks) and reduction of the trabecular and cortical bone thickness in the hind limbs as compared to control monoclonal antibodies (p<0.05). While no macroscopic or MRI signs of synovial inflammation were detected, MRI subclinical inflammation of the tendon sheaths was present in mice injected with ACPAs, but not in those injected with control mAb. Semi-quantitative scoring of the inflammatory tendon changes showed significant higher values in mice injected with ACPA (median of 1, range 0 to 2) than those injected with control mAb (median of 0, range 0 to 1).

Conclusion: We show that ACPA induces pain-like behavior, bone loss and tendon sheath inflammation in mice, a model that mimics the preclinical state of ACPA positive RA.

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