Background: Rheumatoid arthritis (RA) has been associated with increased cardiovascular (CV) risk and metabolic changes.
Objectives: We wished to determine how the Janus kinase (JAK) inhibitor tofacitinib influences vascular pathophysiology and metabolites of the arginine and methionine-homocysteine pathways.
Methods: Thirty RA patients with active disease were treated with either 5 mg bid or 10 mg bid tofacitinib and evaluated at baseline and after 6 and 12 months. We determined DAS28, CRP, IgM rheumatoid factor (RF) and anti-cyclic citrullinated peptide (aCCP) levels. We assessed brachial artery flow-mediated vasodilation (FMD), carotid intima-media thickness (IMT) and pulse-wave velocity (PWV) by ultrasound. We also determined plasma L-arginine, L-citrulline, L-ornithine, inducible nitric oxide synthase (iNOS), asymmetric (ADMA) and symmetric dimethylarginine (SDMA), L-N-monomethyl-arginine (L-NMMA), cysteine, homocysteine, and methionine levels.
Results: Twenty-six patients completed the study. Tofacitinib treatment maintained FMD and PWV. Ten mg bid tofacitinib significantly increased L-arginine, L-ornithine, iNOS and methionine levels after 12 months. Tofacitinib transiently increased L-citrulline and L-NMMA and decreased homocysteine levels after 12 months. Based on L-citrulline, L-ornithine, ADMA and SDMA levels, L-arginine remained highly available for endothelial NO production. Multivariate analysis indicated variable correlations of L-arginine, L-citrulline, ADMA, L-NMMA, homocysteine and methionine with DAS28, CRP, ESR and RF but not with aCCP. Regarding vascular pathophysiology, only PWV and methionine correlated with each other after 12 months.
Conclusion: Tofacitinib suppressed systemic inflammation in RA yielding stabilization of vascular function. It may exert CV protective effects in RA, at least in part, by shifting L-arginine metabolism to high arginine availability and decreasing homocysteine levels.
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POS0384 A NOVEL MECHANISM LINKING MUCOSAL BACTERIA WITH AUTOANTIBODY RESPONSES IN RA: ACETYLATED BACTERIAL LYSATE AS A MODEL ANTIGEN

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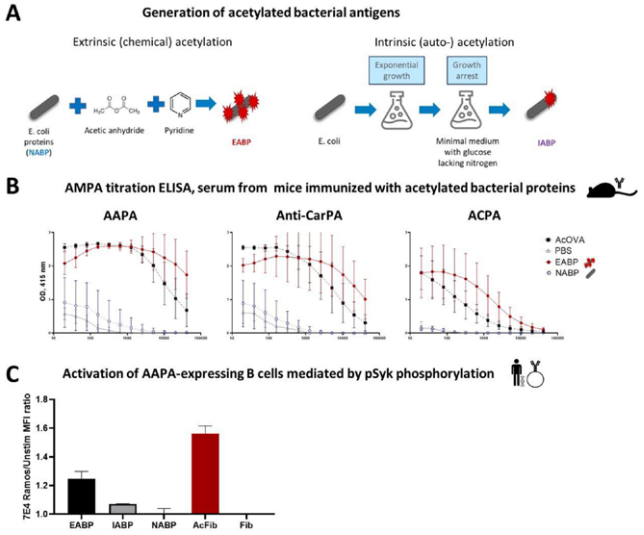
Background: Rheumatoid arthritis (RA) is characterized by autoantibodies against post-translationally modified proteins (AMPA) such as citrullinated, carbamylated and acetylated proteins. Importantly, these antibodies are highly multireactive, as they often recognize more than one of these post-translational modifications. Despite extensive research, the antigens inducing the breach of tolerance remain unknown, although microbial antigens are often suspected. Various bacteria are known to be capable of acetylation, therefore, it is intriguing to know what mechanisms can underlie the breach of tolerance towards acetylated proteins and development of anti-acetylated protein antibodies (AAPA).
Objectives: To investigate whether acetylated proteins of bacterial origin (1) are recognized by human derived AMPA and AMPA expressing B cells; and (2) can induce AMPA development when used to immunize mice.
Methods: Acetylated E. coli proteins were acquired with two separate methods (Figure 1A): by culturing E. coli in a condition promoting auto-acetylation (intrinsically acetylated bacterial proteins, IABP), or by directly acetylating lysate-derived proteins via a chemical reaction (extrinsically acetylated BP, EABP). Acetylated ovalbumin (AcOVA) served as positive control for AAPA induction in mice, non-acetylated BP (NABP) and phosphate buffer saline (PBS) served as negative control. Mice were immunized with these proteins and the resulting antibody response was studied by ELISA. Furthermore, EABP/IABP/NABP were investigated for recognition by human-derived AAPA with ELISA and AAPA-expressing B cells with spleen tyrosine kinase (Syk) phosphorylation assay; acetylated human fibrinogen and native fibrinogen served as positive and negative control.
Results: Repetitive immunization of mice with EABP resulted in an AMPA response recognizing acetylated, carbamylated and citrullinated proteins. AMPA titers in these mice exceeded the titers in the positive control mice immunized with AcOVA and were substantially higher than in the NABP-immunized mice (Figure 1B). Human-derived monoclonal AAPA recognized EABP and IABP (not shown). B cell activation (measured by Syk phosphorylation) assay indicated that AAPA expressing B cells recognized EABP and (to a lesser extent) IABP, but not NABP (Figure 1C).
Conclusion: Acetylated bacterial proteins are potent antigens that can induce cross-reactive AMPA responses in mice and they are recognized by human AAPA. This suggests that acetylated bacterial proteins could possibly be involved in the breach of tolerance in RA.



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POS0385 DURING DEVELOPMENT OF RHEUMATOID ARTHRITIS, INTERMETATARSAL BURSITIS MAY OCCUR BEFORE CLINICAL JOINT SWELLING: A LARGE MRI STUDY IN PATIENTS WITH CLINICALLY SUSPECT ARTHRALGIA

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Background: Inflammation of the synovial lining is a hallmark of rheumatoid arthritis (RA). A synovial lining is not only present at synovial joints and tendon sheaths but also at bursae. Inflammation of the synovium-lined intermetatarsal bursae in the forefoot, intermetatarsal bursitis (IMB), was recently identified with MRI. It is specific for early RA and present in the majority of RA patients at diagnosis. During development of RA, MRI-detectable subclinical synovitis and tenosynovitis often occur before clinical arthritis presents. Whether IMB is also present in a pre-arthritis stage is unknown.
Objectives: To assess the occurrence of IMB in patients with clinically suspect arthralgia (CSA) and its association with progression to clinical arthritis in a large MRI-study.
Methods: We studied 524 consecutive patients presenting with CSA. CSA was defined as recent-onset arthralgia of small joints that is likely to progress to RA based on the clinical expertise of the rheumatologist. Participants underwent unilateral contrast-enhanced 1.5T MRI of the forefoot, metacarpophalangeal (MCP) joints and wrist at baseline. Thereafter patients were followed for detection of clinical arthritis, as identified at physical joint examination by the rheumatologist. Baseline MRIs were evaluated for IMB at all 4 intermetatarsal spaces. Also synovitis, tenosynovitis and osteitis were

assessed in line with the RA MRI scoring system (summed as RAMRIS-inflammation). Both IMB and RAMRIS-inflammation were dichotomised into positive/negative using data from age-matched symptom-free controls as a reference. Cox regression analysed the association of IMB with progression to clinical arthritis; multivariable analyses were used to adjust for RAMRIS-inflammation which is known to associate with progression to clinical arthritis. Analyses were repeated stratified for ACPA-status, since ACPA-positive and ACPA-negative RA are considered separate entities with differences in pathophysiology.

Results: The baseline MRIs showed ≥1 IMB in 35% of CSA-patients. Patients with IMB were more likely to also have synovitis (OR 2.5 (95%CI 1.2–4.9)) and tenosynovitis (8.9 (3.4–22.9)) on forefoot MRI, but not osteitis (0.9 (0.5–1.8)). Patients were followed for median 25 months (IQR 19–27). IMB-positive patients developed clinical arthritis more often than IMB-negative patients (HR 3.0 (1.9-4.8)). This association was independent of RAMRIS-inflammation (adjusted HR 2.2 (1.4–3.6)). In stratified analyses, IMB was more frequent in ACPA-positive than in ACPA-negative CSA (68% vs. 30%, p<0.001). Moreover IMB predicted clinical arthritis development in ACPA-positive CSA (HR 2.5 (1.1–5.7)) but not in ACPA-negative CSA patients (1.0 (0.5–2.2)).

Conclusion: One-third of CSA patients have IMB. IMB is frequently present in conjunction with subclinical synovitis and tenosynovitis. It precedes the development of clinical arthritis, and in particular the development of ACPA-positive RA. These results reinforce the notion that not only intra- but also juxta-articular synovial inflammation is involved in the development of RA.

Disclosure of Interests: None declared

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POS0386 EVOLUTION OF ANTI-MODIFIED PROTEIN ANTIBODY RESPONSES CAN BE DRIVEN BY CONSECUTIVE EXPOSURE TO DIFFERENT POST-TRANSLATIONAL MODIFICATIONS

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Background: Besides anti-citrullinated protein antibodies (ACPA), rheumatoid arthritis patients (RA) often display autoantibody reactivities against other post-translationally modified (PTM) proteins, more specifically carbamylated and acetylated proteins. Immunizing mice with one PTM results in an anti-modified protein antibody (AMPA) response recognizing multiple PTMs. Furthermore, human AMPA, isolated based on their reactivity to one PTM, cross-react with other PTMs at the monoclonal and polyclonal level. However, it is unclear whether the AMPA reactivity profile is "fixed" in time, or whether consecutive exposure to different PTMs can shape the evolving AMPA-response.

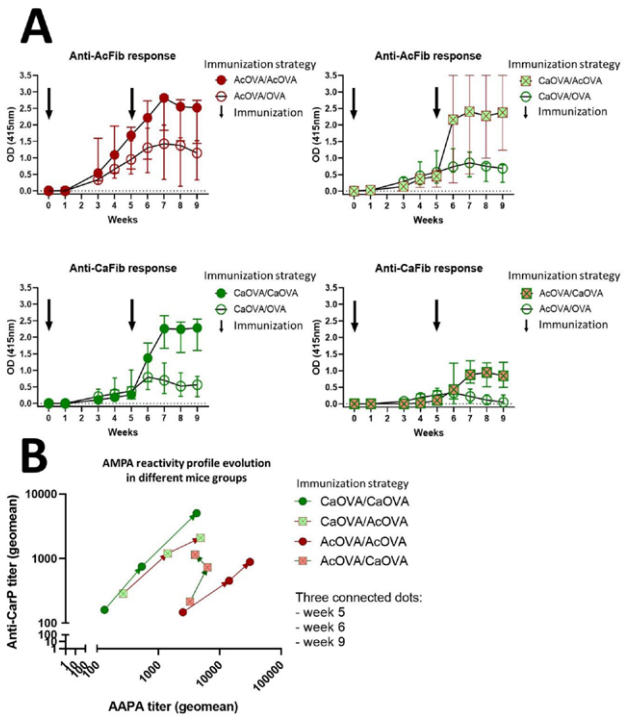
Objectives: To investigate the evolution of the AMPA response in mice with controlled exposure to PTMs as well as in AMPA-positive humans.

Methods: Mice were immunized with acetylated (or carbamylated) protein (ovalbumin) twice or cross-immunized with an acetylated and then a carbamylated protein (or vice versa) and their serum was analyzed for AMPA responses with ELISA using a different backbone protein (fibrinogen) bearing the same modifications. Longitudinally collected serum samples of human individuals at risk of RA and with early RA were tested to investigate the evolution of the AMPA responses in humans.

Results: Mice immunized twice with either solely acetylated or solely carbamylated ovalbumin (AcOVA or CaOVA) developed reactivity against both acetylated and carbamylated antigens. Irrespective of the PTM used for the first immunization, a booster immunization with the other PTM resulted in increased titers to the second/booster PTM (Figure 1A), suggesting that immunization with a defined PTM-antigen leads to the generation of anti-PTM memory B cells able to cross-recognize other PTMs. Furthermore, immunizing with CaOVA and boosting with AcOVA (or vice versa) skewed the overall AMPA-response profile towards a relatively higher reactivity against the "booster" PTM (Figure 1B). Human data also illustrated dynamic changes in AMPA reactivity profiles in both individuals at risk of RA and in early RA patients (not shown).

Conclusion: The relationship between different reactivities within the AMPA response is dynamic. The initial exposure to a PTM antigen induces cross-reactive response that can be boosted by the same or other PTMs. The overall reactivity pattern can be skewed by subsequent exposure to other PTMs. These data might explain temporal changes in the reactivity profile of the AMPA response and point to the possibility that the PTM responsible for the initiation of the AMPA response may differ from the PTM predominantly recognized later in time.

Cross-boosting and skewing of AMPA responses in immunized mice



Disclosure of Interests: None declared

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POS0387 ACPA STATUS CORRELATES WITH DIFFERENTIAL IMMUNE PROFILE OF RHEUMATOID ARTHRITIS PATIENTS

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Background: Rheumatoid arthritis (RA) is a progressive erosive autoimmune disease that affects 1% of the world population. Anti-citrullinated protein autoantibodies (ACPA) are routinely used for the diagnosis of RA, however 20-30% of patients are ACPA negative. ACPA status is a delineator of RA disease endotypes with similar clinical manifestation but potentially different pathophysiology. Elucidating the underlying mechanisms of disease pathogenesis could inform a treat to target approach for both ACPA-positive and ACPA-negative RA patients.

Objectives: To identify peripheral blood and synovial tissue immune population differences that associate with RA disease endotype.

To identify unique RA patient synovial tissue gene signatures and enriched pathways that correlate with ACPA status.

Methods: Detailed high dimensionality flow cytometric analysis with supervised and unsupervised algorithm analysis of ACPApos and ACPAneg RA patient peripheral blood and synovial tissue single cell suspensions. *Ex vivo* peripheral blood and synovial tissue T cell stimulation and cytokine production characterisation. RNAseq analysis with specific pathway enrichment analysis of APCApos and ACPAneg RA patient synovial tissue biopsies.

Results: Detailed profiling based on high dimensionality flow cytometric analysis of key peripheral blood and synovial tissue immune populations including B cells, T follicular helper (Tfh) cells, T peripheral helper cells (Tph) and CD4 T cell proinflammatory cytokine responses with supervised and unsupervised algorithm analysis revealed unique RA patient peripheral blood B cell and Tfh cell profiles. ACPApos RA patients were characterised by significantly (*P=0.03) increased frequency of Tfh ($CXCR5^+CD4^+$) cells and distinct clustering influenced by increased switched (IgD^-CD27^+) and DN (IgD^-CD27^-) memory B cells compared to APCAneg RA patients. Surprisingly synovial tissue B cell subpopulation distribution was similar between ACPAneg and ACPApos RA patients, with significant accumulation of switched and double negative memory B cells, highlighting a key role for specific B cell subsets in both disease endotypes. Interestingly, synovial tissue CD4 T cell