ANCAs. Antigen discovery to determine the targets of these recombinant plasmablast antibodies is ongoing.
Conclusions: Plasmablasts from patients with GPA in the RAVE trial were sequenced and antibody repertoires were generated. Clonal families of plasmablasts, including those expressing antibodies possessing shared CDR sequences across multiple patients, were identified and recombinantly expressed. None of the 24 antibodies bound to PR3, suggesting these are not ANCAs and are potentially novel autoantibodies. Identification of the antigen targets of these antibodies is ongoing.
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SAT0018 EFFECTS OF ANTI-TNF ALPHA THERAPY ON B CELLS IN RHEUMATOID ARTHRITIS (RA) PATIENTS

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Background: Our laboratory has previously characterized defects in humoral B cell responses of aged mice and humans. These defects include: the reduction in activation-induced cytidine deaminase (AID), required for the generation of optimal antibody responses, and the reduction in the percentage/number of the subset of switched memory B cells (1). AID and switched memory B cells have been proposed to be effective predictive biomarkers of vaccine responses (2). Moreover, we have shown that aging is characterized by increased systemic inflammation which induces intrinsic B cell inflammation, measured by intracellular (ic) TNF-α, and this significantly decreases the capacity of the same B cells to make protective antibodies in response to vaccination (3). Other marker of B cell intrinsic inflammation is micro-RNA (miR) expression, particularly miR-16 and miR-155, which is increased in elderly B cells and negatively correlated with B cell function (4).
Objectives: Our goal for this study was to evaluate B cell phenotype and function in RA patients treated Methotrexate (MTX), alone or together with anti-TNF-α. We hypothesized that patients treated with anti-TNF-α will show improved B cell function due to reduction in icTNF-α.
Methods: We recruited 9 RA patients, 5 patients on MTX and 4 on MTX/anti-TNF-α. We measured the relevant B cell subsets in blood (Naïve, switched memory, IgM memory and late memory) by flow cytometry. Staining was performed with antibodies specific for CD19, CD27 and IgD. In addition, we isolated blood B cells using magnetic beads, and measured the expression of miR-16 and miR-155 on blood B cells by qPCR.
Results: Preliminary data showed that the percentages of switched memory (IgD-CD27+) B cells are significantly higher ($p<0.003$) in patients undergoing MTX/anti-TNF-α therapy. We also observed a significant decrease in naïve B cell percentages ($p<0.028$). Preliminary results also showed a decrease in the mRNA expression of both miR-16 and miR-155 in patients on combination therapy compared to MTX alone.
Conclusions: These results support the hypothesis that therapy with anti-TNF-α is beneficial for improving B cell function in RA patients, as compared to MTX therapy alone. Future experiments will seek to evaluate intrinsic B cell TNF levels in these patients and correlate them with measurements of B cell function. Treatment with anti-TNF-α may be able to block the excessive amounts of systemic TNF-α and in turn B cell intrinsic TNF-α which could improve the antibody responses and the risk of infections in RA patients undergoing therapy.
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SAT0019 ESTROGEN INFLUENCES THE SIALYLATION PROFILE AND INFLAMMATORY PROPERTIES OF ANTIBODIES – A POTENTIAL EXPLANATION FOR THE SEX DIFFERENCES AND INCREASED RISK FOR RA IN POSTMENOPAUSAL WOMEN

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Background: Rheumatoid arthritis preferentially affects women. RA has its peak over 50 years coincidencing with the decrease in sex hormones in menopause. Recently, the transition from asymptomatic autoimmunity to RA has been shown to essentially depend on the glycosylation status of antibodies affecting the binding affinity to Fc gamma receptors[1]. Hence a decrease in the sialylation of antibodies resulting from a decrease in the activity of the sialylation enzyme β-galactoside α2,6-sialylltransferase (St6Gal1) was shown to trigger the onset of RA.
Objectives: To test whether estrogen influences the glycosylation status of antibodies and St6Gal1 expression explaining why postmenopausal women are particularly prone to develop RA
Methods: In the experimental part we tested the influence of estrogen on antibody glycosylation and St6Gal1 expression. Ovariectomized mice, which were either left without estrogen supplementation or were supplemented with estrogen (hormone replacement), were immunized with ovalbumin (OVA) to induce antibody production. Immunoglobulin G (IgG) levels were analyzed by ELISA and the glycosylation of the Fc-part of total and OVA-specific IgG was determined by lectin ELISA and MALDI-TOF, respectively. St6Gal1 expression in plasma cells was determined by RT-PCR and FACS. Inhuman part we measured the effects of estrogen treatment on autoantibody levels and IgG glycosylation in a cohort of postmenopausal RA patients over 2 years[2].
Results: Ovariectomy and loss of estrogens was associated with a lower sialylation of OVA-specific IgG. Conversely estrogen treatment significantly increased the sialylation level of newly formed OVA-specific and totals IgG as well as enhanced the expression of St6Gal1 enzyme in plasma cells suggesting a shift towards an anti-inflammatory pattern of IgG. These results were confirmed with estrogen treated postmenopausal RA patients showing that hormone replacement therapy significantly increased antibody glycosylation, while in a control RA population not exposed to estrogens no such increase in sialylation of IgG was found. Estrogens however, did not influence the CCP autoantibody levels.
Conclusions: These findings indicate that estrogen regulates St6Ga1 and increases the glycosylation of IgG. Lack of estrogen decreases IgG glycosylation and results in pro-inflammatory properties of IgG which may explain the increase prevalence of RA in postmenopausal women.
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SAT0020 THE CITRULLINOME IN TISSUE AND BIOFLUIDS OF HUMAN AND MOUSE ORIGIN

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Background: Protein citrullination is fundamental to several essential processes in apoptosis and antimicrobial defense, however, also linked to multiple pathogenic endpoints. This post-translational modification (PTM), by conversion of arginine to citrulline residues, is mediated by peptidylarginine deiminase (PAD) enzymes found in specific cells and tissues. In polymorphonuclear cells (PMNs) these enzymes enable NETosis, a specialized form of programmed necrosis and the formation of NETs (neutrophil extracellular traps). Also, these enzymes are expressed in the synovium of patients with rheumatoid arthritis (RA) thereby triggering the production of autoantibodies against citrullinated proteins (ACPAs).
Objectives: Our objective was to optimize methodology for characterization of this PTM and determine the citrullinome in tissue and biofluids of human and mouse origin in clinical relation to rheumatoid arthritis (RA), osteoarthritis (OA), Spondyloarthritis (SpA) as well as presence of ACAPs and NETs
Methods: Synovial fluid (SF) and plasma was collected from patients diagnosed with RA, OA, SpA (n=120). Inflammation levels patients were characterized with plasma C-reactive protein (CRP), and circulating anti-CCP levels as well as 10 most relevant proinflammatory cytokines. Intestinal tissue (colon mucosa) from RA patients (n=10) and joint lysate from collagen-induced arthritis mouse model (n=24). All samples were analyzed by citrulline specific sample preparation and high-end mass spectrometric analysis [1,2]. Follow-up studies were performed by multiple techniques including confocal microscopy and cell-free DNA measurement.