Background: Current treatment approaches for autoimmune conditions comprise primarily of systemic immunosuppressants or cytokine blockade. The concentration of therapeutic molecules to the tissues that are the sites of autoimmune and inflammatory diseases is a promising approach with the potential to induce therapeutic benefit and avert risks associated with systemic immunotherapies. Pandion Therapeutics is developing a bifunctional antibody platform that can drive localized immune modulation by combining a "tether antibody" that targets a tissue of choice and an "effector end" that activates specific regulatory immune pathways to restore immune-homeostasis.

Objectives: Here we report the engineering of a skin-tethered PD-1 agonist and a skin-tethered CD39 that inhibit T cell activation and function and deplete local ATP, respectively, modulating different arms of the immune system in a tissue specific manner.

Methods: Biophysical assays were performed to characterize Skin-tethered immune effectors for drug-like properties and *in vitro* and *in vivo* assays for target binding, cellular activity and tissue specific-localization. Moreover, these bifunctional molecules were tested in pathway-relevant preclinical models such as Vitiligo and Contact Hypersensitivity.

Results: Biophysical characterization of the bifunctional molecules showed desired drug like properties including specificity, stability, and manufacturability. The skin tethered bifunctionals showed effector activity in *in vitro* assays and selectively localized to the skin. Skin localization strikingly correlated with a tether-dependent efficacy compared to a non-tether control.

Conclusion: We believe that this therapeutic approach has the potential to drive the resolution of cutaneous inflammation, providing an opportunity for developing new targeted therapies for autoimmune and inflammatory skin diseases.

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AB0020 ACPA ILLUSTRATING THE IMPACT OF IGG FAB-GLYCOSYLATION ON TRANSPLENTAL TRANSFER OF ANTIBODIES AND THEIR BINDING TO THE NEONATAL FC-RECEPTOR (FCRN)

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Background: Fc neonatal receptor (FcRn) is crucial for IgG half-life and transplacental transport. Different sites of IgG carry glycans which may affect binding to FcRn. While the effect of Fc-glycans has been investigated, the impact of Fab-glycosylation (~14% IgG) on IgG-FcRn interaction remains unclear. Anti-citrullinated protein antibodies (ACPA) of rheumatoid arthritis patients exhibit remarkably high Fab-glycosylation (~90%). This makes ACPA an ideal model to investigate how Fab-glycosylation influences IgG-FcRn interaction.

Objectives: To investigate the potential impact of IgG Fab-glycosylation on IgG transplacental transfer and interaction with FcRn.

Methods: To investigate transplacental transport of ACPA and total IgG, serum of ACPA-positive RA patients (mothers) as well as of healthy mothers and their respective newborns was analyzed. IgG Fab- and Fc-glycosylation was investigated with liquid chromatography and mass-spectrometry. Furthermore, ACPA monoclonal IgG were produced and glycoengineered to acquire several variants of the same monoclonal antibody differing only in their glycosylation profile. These glycovariants were then used to investigate the impact of Fab-glycans on the affinity of IgG for FcRn. Surface plasmon resonance (SPR) and affinity chromatography were implemented.

Results: When measured in mothers' serum and cord blood samples, Fab-glycosylation of IgG antibodies was ~20% lower in newborns compared to their mothers, which was observed for ACPA IgG, non-ACPA IgG in RA patients and total IgG of healthy controls (Figure 1). This may indicate that transplacental transfer of Fab-glycosylated antibodies is impaired. SPR results suggested that presence of Fab-glycans slightly lowered the affinity of IgG for FcRn. However, presence of Fab-glycans did not have a significant effect on

the results of FcRn affinity chromatography. Together, these results suggest that Fab-glycans may impair association of IgG with FcRn, while dissociation likely stays intact.

Conclusion: Our results suggest that Fab-glycans inhibit IgG-FcRn binding which negatively affects the transplacental transfer of Fab-glycosylated IgG. The impact of Fab-glycosylation on IgG half-life requires further investigation.

Figure 1.

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AB0021 CHARACTERISATION OF AGE-ASSOCIATED B CELLS IN EARLY, DRUG NAÏVE RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis (RA) is a chronic inflammatory disease of immune dysregulation affecting the joints. While T cells play a recognised role in disease pathogenesis, the presence of autoantibodies years before the clinical onset of disease, and the efficacy of the B cell-depleting therapy rituximab, highlight a pathogenic role for these functionally diverse lymphocytes. A novel subset, termed age-associated B cells (ABCs), are described as CD19^{high} CD21⁺ CD11c⁺, with a high proportion expressing the transcription factor T-bet; they are elevated in murine models of autoimmunity and produce autoantibodies characteristic of autoimmune disease. A detailed characterisation of ABCs in early, drug naïve RA has not yet been conducted.

Objectives: We aimed to characterise peripheral blood (PB) and synovial fluid (SF) ABCs in patients suffering from early drug naïve RA. As a secondary objective we sought to determine whether this population differs between RA patients and age-matched early psoriatic arthritis (PsA; disease controls) and healthy controls.

Methods: Newly presenting early RA and other inflammatory arthritis patients, naïve to immunomodulatory treatment, were recruited from the Newcastle Early Arthritis Clinic. Patients with established RA (≥1 year duration on treatment and age-matched healthy controls were recruited in parallel. B cell subsets (naïve (CD19⁺IgD⁺CD27⁻), switched memory (CD19⁺IgD⁺CD27⁺) and ABC (CD19⁺CD21⁺CD11c⁺)) in PB and SF were characterised by flow cytometry. FACS sorted PB B cell subset gene expression profiles were assessed using a customised NanoString nCounter Human Immunology v2 Panel.

Results: Transcriptionally, ABC differed from both naïve and switched memory B cells. In keeping with published studies ABCs had an activated memory phenotype, exemplified by elevated expression of CD69, CD80, and CD86, as well as Ki67, HLA-DR, IgG and T-bet. Interestingly, ABC had high expression of the Fc Receptor Like (FcRL) family (FcRL1-5), and an inflammatory homing profile (high levels of CXCR3 and low levels of CXCR4 and CXCR5). We found no difference in the proportion of PB ABC between RA patients and control groups, and no association of ABC frequencies with age. Focussing on RA ABCs specifically, we observed elevated ABC frequencies in females, but no association with disease activity. In keeping with their chemokine receptor profile, cross-sectional analysis also demonstrated ABC enrichment in SF compared to PB, with SF frequencies higher in established than early disease. The transcriptome of ABCs from early RA patients differed from both age-matched disease controls and healthy donors (Figure 1 next page).

Conclusion: These data demonstrate that ABCs have a unique, activated, class-switched proliferative phenotype that is transcriptionally distinct from switched memory and naïve B cells. Interestingly, at the transcriptome level early RA ABCs differ to their counterparts in health and other inflammatory arthritides, suggesting they may differ functionally and contribute to pathogenesis. Of note,

their high expression of MHC class II (HLA-DR) and co-stimulatory (CD80 and CD86) molecules suggests an important antigen-presentation function of ABC, which together with their unique FcRL family expression pattern, warrants further functional characterisation.

Figure 1. Differential gene expression in ABCs from early RA patients compared to early PsA patients (A) and healthy controls (B). A NanoString nCounter Technologies chip was used to assess gene expression. Raw counts were normalised to the housekeeping genes. Sample quality was then assessed using the arrayQualityMetrics package. Gene expression profiles between different donor groups was assessed using the DESeq2 R package. In both hierarchical clustering heatmaps gene expression intensities were log2 transformed and their z-scores are displayed as colours ranging from yellow (low expression) to red (high expression) as shown in the key.

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AB0022 AUTOREACTIVE B CELLS IN RHEUMATOID ARTHRITIS DISPLAY AN ACTIVATED PHENOTYPE OF RECENT ANTIGEN EXPOSURE

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Background: Rheumatoid arthritis, in particular ACPA+ RA, is characterized by frequent disease flares and poor chances to achieve DMARD-free sustained remission. Recently, we have shown that ACPA-expressing memory B cells (MBC) remain in a persistently activated state throughout disease, even in patients in DMARD-induced clinical remission.⁽¹⁾ The reasons why the ACPA B cell response is continuously activated are unknown, as well as why the response does not revert to a resting, 'quiescent' state. We hypothesized that continuous antigen exposure in germinal centres drives ACPA B cell activation, leading to a 'recent germinal centre emigrant' phenotype of these cells in the circulation.

Objectives: To understand whether the activated phenotype of ACPA-expressing B cells could be induced by recent antigen exposure, to thereby discern the processes of immune activation that remain active in patients even in clinical remission and to argue whether these processes could be targets for therapeutic intervention.

Methods: ACPA-expressing B cells were identified in peripheral blood of RA patients by flow cytometry during different stages of disease and characterized by a panel of activation- and germinal centre related markers (CD80, CD86, CD32, CD95, Ki-67). In addition, three healthy donors received a TT booster vaccination. TT-specific MBC were identified in blood at different timepoints (before vaccination and up to 22 weeks after vaccination) and analysed phenotypically over time.

Results: The majority of ACPA-expressing B cells strongly expressed CD95 and the co-stimulatory marker CD80. A part was also positive for the proliferation marker Ki-67 (on average 30%), and most cells downregulated the inhibitory marker CD32. TT-specific MBC adopted a comparable phenotype after booster vaccination, but most markers returned to the pre-vaccination expression level gradually over time. These effects were antigen-dependent because the phenotype of TT-negative B cells remained unchanged. The phenotypic composition of the proliferating ACPA-positive B cell pool most closely corresponded to a stimulation history of 1-2 weeks after antigen exposure. Notably, none of the Ki-67 negative ACPA-specific MBC showed phenotypic quiescence, indicating either a short life-time (in circulation) after antigen encounter or persistent additional factors of activation.

Figure 1. Ki-67 expression on ACPA-specific MBC in RA (A) and on TT-specific MBC in 3 healthy donors before and after booster vaccination (B).

Conclusion: ACPA-expressing MBC phenotypically resemble TT-specific MBC after recent (1-2 weeks) booster vaccination, reflecting the phenotype of recent germinal centre emigrants, and remain activated, whereas TT-specific MBC lose this marker profile over time. These observations suggest that ACPA-expressing MBC either home to tissue or survive shortly in the circulation, or that additional factors drive or program these cells to persistent activation. Transcriptomic profiling and analysis of the homing marker profile may help to answer these questions. Furthermore, it will be important to understand the association of persistent activation of ACPA-expressing B cells in clinical remission and the risk for disease flares upon treatment discontinuation.

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AB0023 DENDRITIC CELLS AS A PROMINENT MARKERS OF AUTOIMMUNE DISEASES

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Background: Dendritic cells (DCs) are known to contribute to the pathogenesis of different autoimmune diseases. It is clear nowadays the role of DCs in rheumatoid arthritis (RA) but not well investigated in Axial spondylitis (AxSpA). DCs are a heterogeneous population and can be divided into groups: myeloid (mDCs) and plasmacytoid (pDCs). DCs can induce both immune response and tolerance.

Objectives: To investigate the subpopulations of peripheral blood myeloid and plasmacytoid DCs in patients with early stage of RA (duration of illness up to 12 months) and AS.

Methods: The study include sixty five patients with early forms of diseases including 55 patients with RA and 10 patients with AxSpA. Diagnosis RA was established according ACR/EULAR criteria (2010). Diagnosis AxSpA was established according ASAS criteria. All patients received conventional synthetic DMARDs. Thirty patients with osteoarthritis (OA) used as a control group. Analysis of the content of the B-lymphocytes, myeloid and plasmacytoid DCs was carried out by flow cytometry. B-lymphocytes, subtypes of peripheral blood DCs were characterized by the following phenotypes: myeloid DCs (CD3-CD14-CD19-HLA-DR + CD11c + CD123-), plasmacytoid DCs (CD3-CD14-CD19-HLA-DR + CD11c-CD123 +), B-lymphocytes (CD19 +). Analyses were performed before treatment and after 3 and 6 months.

Results: Patients with early RA are characterized by significant evaluation of the population of myeloid DCs in comparison of patients with osteoarthritis (25.3% vs 21.5, $p=0.005$). Furthermore, the difference was found in the number of cells with the phenotype B-lymphocytes: 5.7% vs 3.1%, $p=0.0007$. No significant differences were observed in the number of plasmacytoid DCs. After 3 and 6 month of observation we detected reducing amount of myeloid DCs 26.7% vs 20.1% vs 16.4% respectively. Disease activity according to DAS28 dropped to low (4.32 to 3.06, $p=0.03$). Patients with AxSpA are characterized by a lower mDCs levels in compared with RA (19.3% vs 26.7, $p=0.07$). After 6 month of investigation we detected decreasing mDCs (19.3% to 14.2%, $p=0.05$). The percent of pDCs were constant and did not differ from the level of healthy donors.

Conclusion: The data obtained indicate that early form of rheumatic diseases namely rheumatoid arthritis and axial spondylitis have the common features such as the dominance of mDCs and their decreasing in reduction of activity of disease.

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