Patients were stratified based on disease activity. Remission was defined as no recorded DAS28-CRP ≥2.4, no swollen joints, no C-reactive protein (CRP) of >3 mg/L, and on a stable DMARD dose and no reported disease flare/loss of remission in the 6 months prior. Non-remission was defined as any other disease activity which does not fulfil the remission definition. Patients on abatacept, or methotrexate monotherapy and healthy volunteers were recruited as comparison groups. A separate cohort of anti-TNF patients (longitudinal cohort) who have been in remission on a stable dose of anti-TNF for ≥6 months and no use of corticosteroids in the last 6 months, was also recruited. Whole blood was obtained prior to dose tapering (dose halving) and at the point of a flare. Whole blood was processed by gradient centrifugation to obtain peripheral blood mononuclear cells (PBMC). PBMC were stained with fluorochrome-conjugated antibodies for multi-parameter flow cytometry. Analysis was performed on live lymphocytes using FlowJo software version 10.8. Two-tailed Mann-Whitney U test or unpaired t-test were used to obtain unadjusted values, analysis of variance (ANOVA) of log-transformed data was used to obtain age-adjusted values, Spearman's rank correlation was used to compare correlation between Temra and CRP.

**Results:** RA patients (36 anti-TNF, 12 abatacept, 16 methotrexate monotherapy) and 14 healthy individuals were recruited. There was a higher proportion of CD4 (age-adjusted p = 0.004) and CD8 Temra (age-adjusted p = 0.0007) in RA patients on anti-TNF with persistent disease activity compared to those who had achieved remission. These differences were confirmed when analysing absolute numbers of CD4 and CD8 Temra. Unexpectedly, the difference in Temra frequency between remission and non-remission RA was not observed in patients treated with methotrexate or abatacept. The median CD4 and CD8 Temra frequencies in RA patients in remission with all treatments studied were similar to healthy individuals. Temra were not observed to increase with age in the anti-TNF, abatacept, or methotrexate cohorts in contrast to previous reports in healthy individuals.2 The frequency of CD4 and CD8 Temra correlated with CRP only in patients on anti-TNF (CD4 Temra Spearman r = 0.5185, p = 0.001, and CD8 Temra Spearman r = 0.5040, p = 0.005).

There was an increase in CD4 (p = 0.003) but not CD8 Temra at 3 months in patients who flared on tapering anti-TNF compared to those who remained in remission (Figure 1).

**Conclusion:** Increased CD4 and CD8 Temra frequency were associated with persistent disease activity in anti-TNF treated patients but not with other DMARD therapies (abatacept and methotrexate). CD4 Temra increased in those who flared on tapering anti-TNF. These results suggest that Temra may play a role in driving persistent disease activity refractory to anti-TNF therapy rather than merely a marker of inflammation.

**REFERENCES:**


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**OP0012**

**INTERFERON-Α IMPLICATES EPIGENIC REPROGRAMMING IN EARLY RA IMPACTS EPIDEMIC RESISTANCE**

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**Background:** An interferon gene signature (IGS) is present in approximately 50% of early, treatment naive rheumatoid arthritis (eRA) patients. We previously demonstrated it negatively impacts on initial disease outcomes.

**Objectives:** To 1) reproduce previous findings demonstrating the harmful effects of the IGS on early RA clinical outcomes; 2) identify which IFN class is responsible (IFN-α, IFN-γ and IFN-λ) and 3) seek evidence that IFN-α exposure contributes to harmful epigenetic footprint at disease onset.

**Methods:** In a large multicentre inception cohort (n=190) of eRA patients (RA-Map TACERA) whole blood transcriptome, IGS (Mxα, IFI44L, OAS1, ISG15, IFI6) and circulating interferons (IFN-α, -γ and -λ) was examined at baseline and 6 months in conjunction with disease activity and clinical characteristics. A separate eRA cohort of paired methyleme and transcriptome from CD4 T and CD19 B cells (n=1 for each) was used to explore any epigenetic influence of the IGS.

**Results:** The baseline IGS reproducibly and significantly negatively impacts on 6-month clinical outcomes. In the high IGS cohort there was increased DAS-28 (p=0.025) and reduced probability of achieving a good EULAR response (p=0.034) at 6-months. In addition, the IGS in eRA is shown for the first time to predominantly reflect raised circulating IFN-α protein, not other classes of IFN and examination of whole blood upstream nucleic acid sensors expression suggest a RNA trigger. Both the IGS and IFN-α significantly fell in parallel at 6 months (p<0.0001), whereas other classes of IFN remained statistically static. There was a significant association with IFN-α and RF titre but not ACPA. Comparison of CD4 T and CD19 B cells between IGS high and low eRA patients demonstrated differentially methylated CpG sites and altered transcript expression of disease relevant genes e.g. PARP9, STAT1, EPTSI1 which was similarly, and persistently, altered 6 months in the separate TACERA cohort. Differentially methylated CpGs implicated altered transcription factor binding in B cells (GATA3, ET31, NFACT2, EZH2) and T cells (p300, HIIF1α) which cumulatively suggested IFN-α induced epigenetic changes promoting increased, and sustained, lymphocyte activation, proliferation and loss of anergy in the IGS high cohort.

**Conclusion:** We validate that the IGS is a robust prognostic biomarker in eRA predicting poor therapeutic response. Its persistent harmful effects may be driven via epigenetic modifications. These data have relevance for other IGS-α states, such as COVID-19, but also provide a rationale for the initial therapeutic targeting of IFN-α signalling, such as with JAKI, at disease onset in stratified eRA subsets.

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OP0013

LOSS OF SYNOVIAL TISSUE MACROPHAGE HOMEOSTASIS PRECEDES RHEUMATOID ARTHRITIS CLINICAL ONSET

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Background: Synovial tissue macrophages significantly contribute to Rheumatoid Arthritis, yet the precise nature/function of macrophage subsets within the inflamed joint remains unexplored.

Objectives: To fully explore the spectrum of distinct macrophage activation states residing within the synovium of RA, at risk and healthy individuals.

Methods: Single-cell synovial tissue suspensions from RA (n=44), IAR (n=5), HC (n=11), PaA (n=11) and OA (n=4) were obtained, and synovial macrophage subsets examined by advanced multiparameter flow cytometric analysis, bulk RNA-sequencing, metabolic and functional assays.

Results: Multidimensional analysis identifies enrichment of CD206+CD163+ synovial-tissue macrophages co-expressing CD40 in the RA joint compared to healthy synovial-tissue, with frequency of CD206+CD163+CD40+ macrophages associated with increased disease activity and treatment response. In contrast, CX3CR1-expressing macrophages which form a protective barrier in healthy synovium are significantly depleted in RA. Importantly, this significant enriched CD40 expression coupled with depleted CX3CR1 expression is an early phenomenon, occurring prior to clinical manifestation of disease in individuals ‘at-risk’ of RA (IAR). RNAseq and metabolic profiling of sorted RA synovial-macrophages identified that this population is transcriptionally distinct, displaying unique inflammatory, phagocytic and tissue-resident gene signatures, paralleled by a biologically stable profile as indicated by NAD(P)H emission. Functionally CD206+CD163+ RA macrophages are potent producers of pro-inflammatory mediators (reversed by CD40-signalling inhibition) and induce an invasive phenotype in healthy synovial-fibroblasts. These findings identify a distinct pathogenic population of synovial-tissue macrophage involved in shaping the immune response in RA. Crucially, this signature is present pre-disease representing a unique opportunity for early diagnosis and therapeutic intervention.

Conclusion: We have identified a novel population of tissue-resident macrophages in the RA synovium which are transcriptionally/metabolically distinct and capable of contributing to disease pathology. Uncovering the molecular patterns and cues that transform this immunoregulatory macrophage population into a dysfunctional inflammatory activation state may provide opportunities to reinitiate joint homeostasis in RA patients.

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OP0014

CTLA4-Ig INDUCES TOLERGENIC PROPERTIES OF MACROPHAGES BY ALTERING CELLULAR METABOLISM

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Background: Dendritic cells (DCs) are well-recognized for their dual role either for T cell activation (1) or for inducing T cells tolerance (2). Their ability to modulate T-cell responses has made them an interesting tool for the immunotherapy of autoimmune diseases (3). Cytotoxic T lymphocyte antigen 4 (CTLA4) is a negative co-stimulatory molecule, which binds to CD80/CD86 on DCs. CTLA4 induces its immunoregulatory function through trans-endocytosis resulting in impaired co-stimulation (4), or through the induction of indoleamine-pyrole 2,3-dioxygenase (IDO) enzyme (5). Moreover, it has been demonstrated that CTLA4 impairs the autophagic machinery of DCs and therefore suppresses DC inflammatory function (6).

Methods: In this study we focused on tolerogenic DCs (tDCs) and we applied CTLA4-Ig as a tool to induce them. We aim to assess the immunoregulatory potential of CTLA4-mediated tDCs and to investigate thoroughly the intracellular pathways that are involved in the induction of tolerance.

Objectives: In this study we focused on tolerogenic DCs (tDCs) and we applied CTLA4-Ig as a tool to induce them. We aim to assess the immunoregulatory potential of CTLA4-mediated tDCs and to investigate thoroughly the intracellular pathways that are involved in the induction of tolerance.

Methods: Healthy human monocytes were isolated from peripheral blood and differentiated into monocyte-derived dendritic cells (DCs). After 6 days, immature DCs activated with LPS were treated with CTLA4-Ig or IgG control for 18 hours. The anti-inflammatory function of DCs was validated using RT-PCR and flow cytometry and DCs proceeded to RNA sequencing. The metabolic pathways were studied using a Seahorse bioanalyzer.

Results: CTLA4-Ig-treated DCs showed significantly decreased HLA-DR, CD80/CD86 expression as compared to IgG-treated cells (n=4, p=0.0294, n=5 p=0.0079). Moreover, IL6 and TNFα mRNA expression, hallmarks of inflammatory cytokines secreted by DCs, was reduced upon CTLA4-Ig (n=5, p=0.0079). In addition, CTLA4-Ig-treated DCs showed significantly decreased HLA-DR, CD80/CD86 expression as compared to IgG-treated cells (n=4, p=0.0294, n=5 p=0.0079). Moreover, IL6 and TNFα mRNA expression, hallmarks of inflammatory cytokines secreted by DCs, was reduced upon CTLA4-Ig (n=5, p=0.0079).

Conclusion: In this study we focused on tolerogenic DCs (tDCs) and we applied CTLA4-Ig as a tool to induce them. We aim to assess the immunoregulatory potential of CTLA4-mediated tDCs and to investigate thoroughly the intracellular pathways that are involved in the induction of tolerance.


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OP0015

PROINFLAMMATORY MONOCYTES AND MACROPHAGES IN SYNOVIAL FLUID AND BURSAL TISSUE OF PATIENTS WITH POLYMYALGIA RHEUMATICA: POTENT PRODUCERS OF IL-6 AND GM-CSF

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Background: Polymyalgia rheumatica (PMR) is a common, rheumatic inflammatory disease. Inflammation of bursae and tendon sheaths is a characteristic finding