Title:

Mechanistic impasses: tofacitinib treatment ameliorates GM-CSF-driven macrophage inflammation

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Running title:

Tofacitinib reprograms GM-Mϕ

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Granulocyte-macrophage colony stimulating factor (GM-CSF, CSF2) has long been recognized as a therapeutic target in inflammatory diseases such as rheumatoid arthritis (RA), despite the complication of its steady-state role in the lung. The receptor for GM-CSF is composed of an α-chain specific to GM-CSF and a β-chain that is common to IL-3 and IL-5. Signaling is principally via the PI3K-Akt, JAK-STAT, NFκB and ERK pathways, and it is the relative contributions of these pathways that are recognized for the potent effect of GM-CSF on macrophages (Mϕ), which polarizes them to an "M1-like" phenotype and contributes to disease activity. Satoeya et al. (1) reported that tofacitinib (a JAK inhibitor) can therapeutically reprogram GM-CSF-primed macrophages (GM-Mϕ) derived from RA patients.

The authors firstly demonstrate that GM-Mϕ form a distinct inflammatory phenotype in RA, revealing an IL-1β^{+}S100A^{+}HIF-1^{+}IL-10^{lo}NFIL-3/6^{lo} profile with mitochondrial oxidative stress and fragmentation. Mϕ activated by TLR ligands and pro-inflammatory mediators such as GM-CSF increase levels of mitochondrial reactive oxygen species (ROS), contributing to oxidative stress, which strengthens their inflammatory phenotype(2). The mitochondria in these cells often have a greater degree of fragmentation which further increases ROS accumulation. The resulting Mϕ phenotype contributes to a sustained inflammatory response, as seen in RA.

Despite the success of biologics in treating RA and other inflammatory autoimmune diseases, a significant minority of patients do not respond adequately or lose response over time. Satoeya et al.(1) refer to previous research (3, 4) indicating that anti-TNF and anti-IL-6R did not decrease the gene expression of GM-CSFRα/β or GM-CSF in the RA synovium, and that these genes are correlated with disease activity and measures of Mϕ accumulation in this tissue. These data suggest that GM-Mϕ are refractory to the effects of these biologics and indicate another form of therapeutic targeting might be ameliorative.

The dual role that mitochondria play in metabolism and, through the contribution to ROS, the inflammatory process presents an opportunity to reduce chronic inflammation by the modulation of metabolic activity. Multiple options have been proposed for therapeutic targeting of the immune-metabolic axis(5). However, in Satoeya N et al., metabolic interventions were ineffective in reversing the inflammatory or metabolic dysfunction observed in GM-Mϕ. Complex I inhibition and glucose-uptake blockade had a muted impact on inflammatory cytokine production and mitochondrial dynamics. In contrast, tofacitinib

reduced the production of pro-inflammatory mediators while up-regulating regulatory and pro-repair gene expression in GM-Mϕ derived from RA patient leucocytes; a similar effect was observed using GM-CSF-stimulated synovial explants. This effect was achieved in part by tofacitinib effectively downregulated GM-CSFR expression and blocked STAT5 signalling. These changes were associated with reduced inflammatory gene expression and reprogrammed the GM-Mϕ into a more regulatory phenotype, while restoring mitochondrial health. Lastly, the authors used an adenoviral vector to deliver sustained GM-CSF production to the murine intra-articular space to model arthritis in vivo. Tofacitinib reduced the resulting joint inflammation, confirming its ability to correct GM-CSF–dependent pathology.

Beyond the five central findings (Figure 1), the study underscores several details that clarify why the GM-CSF/STAT5 axis is important in RA. First, RA endotypes are heterogeneous, with distinct cellular and molecular drivers, which complicates drug selection and can result in variable responses to biologics. Second, the work assesses samples from both blood and synovial compartments in human RA patients and applies those findings to a GM-CSF-specific animal model of articular inflammation, strengthening its relevance as a therapeutic target. Third, mitochondrial dynamics of GM-Mϕ are biased towards fragmentation and, as their inflammatory activity decreases, mitochondrial morphology and function improve, highlighting the organelle's status as a readout of Mϕ function. Fourth, when the GM-Mϕ phenotype is pharmacologically redirected, regulatory features are restored; tofacitinib promotes a Mϕ phenotype marked by pro-resolution gene expression alongside improvements in oxidative stress and mitochondrial function, suggesting immunoregulatory reprogramming rather than mere cytokine suppression. Taken together, the data suggest that commonly used cytokine-blocking biologics (e.g. anti-TNF, anti-IL-6R) and metabolic monotherapies may be insufficient to remodel the GM-Mϕ phenotype. In contrast, targeting STAT5 via tofacitinib elicits a more comprehensive effect that encompasses receptor down-modulation, signalling blockade, metabolic correction, and organelle repair.

Biologic therapies for auto-immune disease have improved outcomes for millions of patients worldwide. Of the three biologics relevant to Satoeya N et al, (α-GM-CSF, α-IL-6R, α-TNF) much attention had been paid to the potential for GM-CSF blockade to potentiate pulmonary alveolar proteinosis (PAP). Mice lacking expression of the ligand or either alpha or beta chain of the receptor develop PAP in the neonatal stage(6); these symptoms reproduce the human condition acquired by hereditary or autoimmune processes. Nonetheless,

development of GM-CSF blocking biologics has proceeded, with careful management of the therapeutic window between reduction of disease and complete neutralisation of GM-CSF. Several clinical trials (referenced in (7)) have demonstrated that GM-CSF blockade is effective in reducing disease activity in RA without the development of PAP, but it has been shown to be less effective than tofacitinib(7). Clinical trials for the blockade of CCL17, a mediator induced by GM-CSF, have had only modest effects in early proof-of-concept studies(8) and have failed to meet primary endpoints in phase-2 trials (NCT05838742, NCT05838755) which were terminated; these outcomes demonstrate the difficulty in targeting the effects of GM-CSF.

Despite the discovery of TNF, IL-6 and GM-CSF in RA synovial fluid at approximately the same time, the development and commercialization of biologics to block their activity have not progressed equally. TNF and IL6R blockade, along with therapies such rituximab and abatacept, have established a high threshold of efficacy that few new therapies can meet or exceed in the way that JAK inhibitors have done. However, as only ~15–40% of RA patients treated with α-TNF/methotrexate combination therapy achieve remission (DAS-28<2.6) within 6-12 months, and 20-40% of initial responders lose response within 12 months(9, 10), new treatment options are required to address unmet need in patient subsets, as is a better understanding of how to match current treatments to the patients most likely to benefit.

Figure 1. Therapeutic targeting of GM-CSF-primed macrophages.

Satoeya et al. define five key features of GM-CSF primed macrophages (GM-Mϕ) (clockwise from top). GM-CSF reprogrammed cells from blood form patients with RA to generate a pro-inflammatory IL-1β^{+}S100A^{+}HIF-1^{+}IL-10^{lo}NFIL-3/6^{lo} phenotype, with mitochondrial stress. Beyond a reduction in IL-1β, blockade of TNF and IL-6R had only nominal effects on pro-inflammatory GM-Mϕ. Metabolic targeting of glycolysis (Gly) or oxidative phosphorylation (with 2-deoxy glucose and IACS-010759, respectively) had a limited impact on inflammatory cytokine production and mitochondrial dynamics. Conversely, tofacitinib (TOF) blocked STAT5 signalling and down-regulated GM-CSFR expression; consequently, the Mϕ phenotype switched from pro-inflammatory to pro-resolution, while mitochondrial homeostasis was restored. Finally, a GM-CSF-induced form of articular inflammation was significantly reduced by TOF.

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Figure 1. Therapeutic targeting of GM-CSF-primed macrophages.