Wnt signaling induces chemokine production and cell migration of circulating human monocytes

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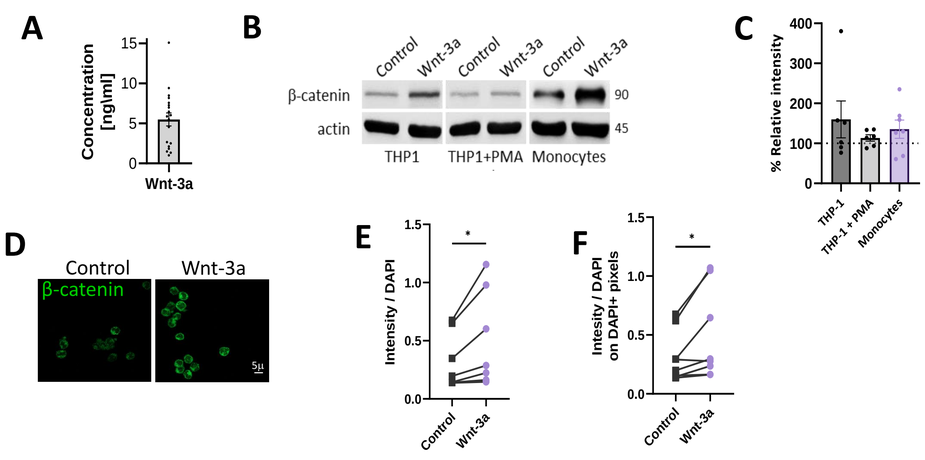
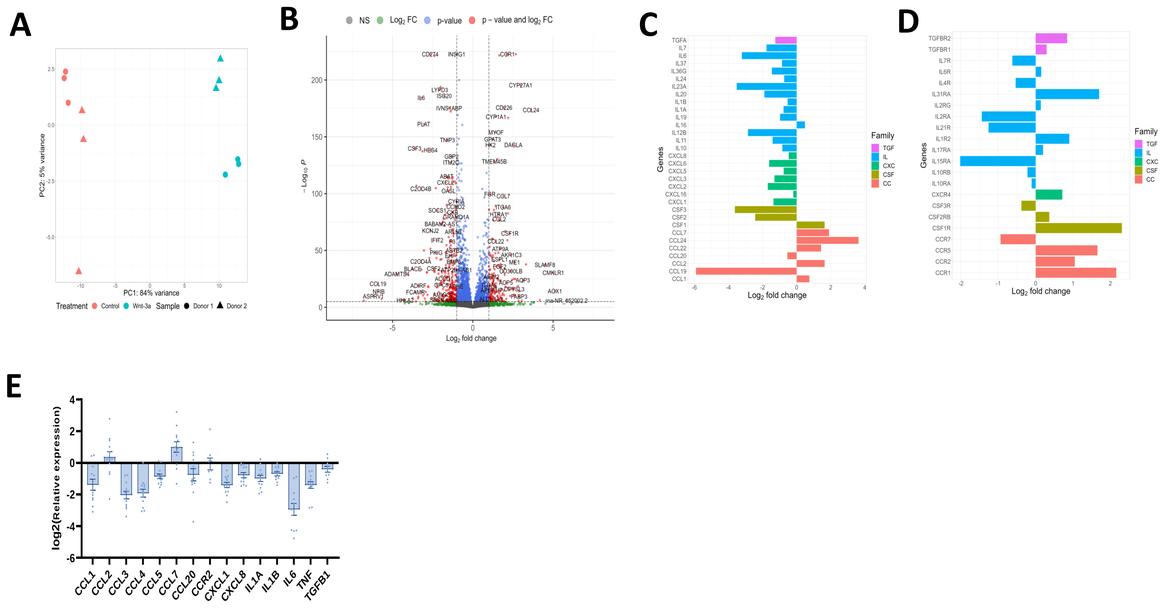
Abstract and Introduction: The β-catenin dependent Wnt signaling pathway is one of the key cascades regulating cellular development and homeostasis. Uncontrolled Wnt signaling is tightly associated with pathological conditions, including inflammation processes and various cancer types. Here, we reveal a link between leukocyte inflammatory responses and canonical Wnt signaling. We show that circulating human primary monocytes express different Wnt signaling components and are susceptible to stimulation by Wnt-3a, which is known to activate the canonical Wnt cascade. This effectively increases β-catenin protein levels, induces cytokine and chemokine secretion, and results in enhanced monocyte migration. Moreover, in monocytes of patients with rheumatic joint diseases, Wnt-3a generates a pattern of cytokine expression different from healthy donor monocytes. Wnt-3a is a potent stimulation factor of monocyte-driven immune processes and these findings have the potential to promote our understanding of inflammatory diseases.

Figure 1: Wnt-3a is present in plasma and induces expression and nuclear accumulation of β-catenin in monocytes



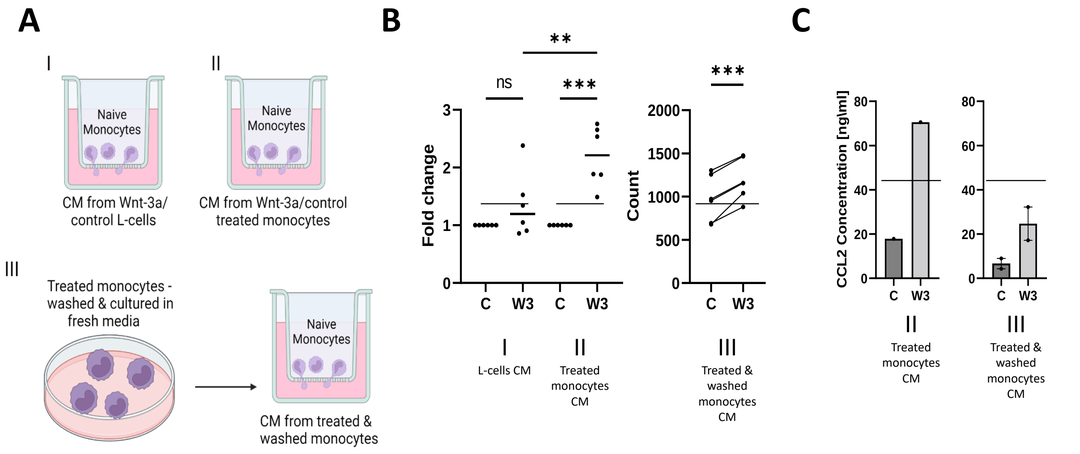
A. Wnt-3a levels were measured by ELISA in human plasma from multiple donors. **B-C**. THP-1 cells (monocyte-like), PMA-treated THP-1 cells (macrophage-like), and primary monocytes were cultured in control or Wnt-3a conditioned media. Cells were lysed, and western blot analysis was conducted (**B**). Band intensity was quantified. The Y-axis represents the band intensity of β-catenin standardized to actin of Wnt-treated cells relative to control-treated cells (**C**). **D-F**. Treated monocytes were stained and visualized using immunofluorescence (**D**). Signal intensity (E) and nuclear localization (**F**) of β-catenin were quantified. Pairs signify individual donors, where each point represents the mean value of at least 5 fields.

Figure 2: Wnt-3a modifies the levels of monocyte-secreted immune transcription and protein levels



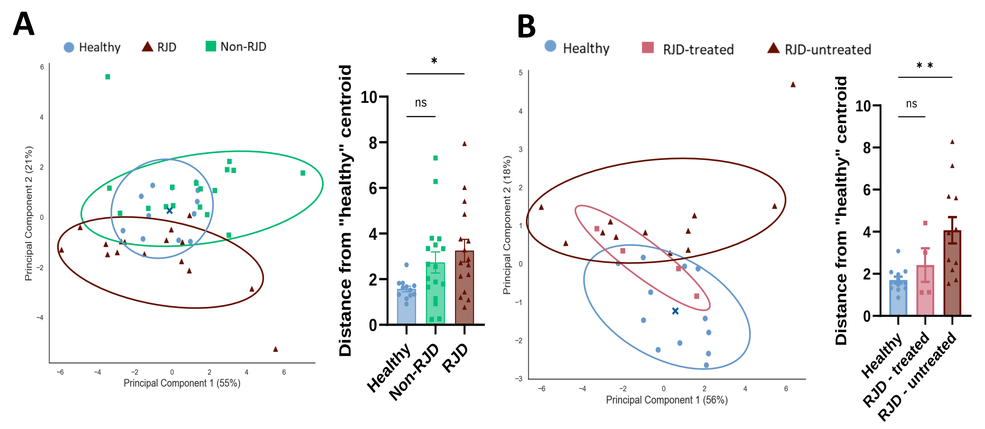
A-D. Monocytes were cultured in control or Wnt-3a conditioned media for RNA sequencing. Principal component analysis (PCA) based on entire gene expression patterns, with batch effect removal to standardize the different donors (**A**). Volcano plot of all genes based on differential expression analysis between control- and Wnt-treated monocytes. Significantly regulated genes are in red on either side (**B**). **C-D**. Summary of Log2 Fold Change of significantly regulated cytokine and chemokine ligands (**C**) and receptors (**D**). **E.** Similarly treated monocytes from multiple blood samples were lysed for RT-qPCR assays of the indicated cytokines and chemokines.

Figure 3: Treating monocytes with Wnt-3a induces cell migration and CCL2 expression



Naïve monocytes were plated in the top chamber of a transwell and exposed to different conditioned media. Cells that crossed the transwell were collected from the bottom well and counted by flow cytometry. **A.** Experimental design: ***I.*** Naïve monocytes were exposed to Wnt-3a/control conditioned media obtained from L-cells. ***II.*** Naïve monocytes were exposed to Wnt-3a/control CM obtained from other monocytes, treated with Wnt-3a/control. ***III.*** Treated monocytes (as in II) were washed and transferred to fresh RPMI medium for an additional hour. Media were collected and used to treat naïve monocytes in the transwell assay. **B.** Flow cytometry counts of cells crossing the transwell barrier. II and III were standardized to the control number (I). **C.** ELISA assays of CCL2 concentration in media used for the indicated experiments.

Figure 4: The inflammatory state of patients with rheumatic joint diseases (RJD) affects monocyte reaction to Wnt-3a stimulus



Principal component analyses (PCA) based on expression of 13 genes in RT-qPCR data of monocytes treated as in Fig. 2E. The blue X represents the centroid of the healthy donor samples. The bar graphs depict the distance of each measurement from the healthy donor centroid. **A.** RJD versus other inflammatory conditions **B.** RJD patients treated with immunosuppressive agents compared to untreated RJD patients.

Conclusions: Our results suggest that monocytes of RJD patients react differently to Wnt-3a stimulus in terms of chemokine and cytokine expression. Most interestingly, following immunosuppressive treatment, the patient monocytes exhibit a more healthy expression of these immune response effectors. Further studies into Wnt signaling in the context of circulating cells and inflammation may provide the basis for a novel concept of circulation signaling pathways that modulate systemic changes and affect core biological processes at sites of inflammation.

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