DO FUNCTIONAL INTERACTIONS BETWEEN MACROPHAGES AND SYNOVIAL FIBROBLASTS DRIVE SYNOVIAL PATHOLOGY OR RESOLUTION OF INFLAMMATION?

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Background: We recently uncovered heterogeneity in human synovial tissue macrophages during inflammation and resolution of inflammation (remission) in rheumatoid arthritis (RA). The transcriptomic profiles of distinct macrophage subpopulations suggest distinct functions, ranging from inflammatory to regulatory. Prior studies propose reciprocal interactions between macrophages and synovial fibroblasts (FLS) exist in the joint; however, the exact contribution of these interactions to synovial pathology or resolution of inflammation remains unknown.

Objectives: The aim of this study was to examine the functional interactions between different macrophage phenotypes and synovial fibroblasts.

Methods: To model different macrophage phenotypes, monocyte-derived macrophages (MDM) were pre-stimulated (16h) with LPS (100ng/ml) to model inflammatory macrophages, or with dexamethasone (Dex, 1 μM) to model regulatory macrophages. After extensive washing, MDM were co-cultured with RA FLS for different periods of time. Prior to co-culture, FLS and MDM were labeled with CellTrace™Violet and CellTrace™Red, respectively. After 24 & 48 hours of co-culture, FLS and MDM were sorted based on positivity for CellTrace™Violet (FLS) or Red (MDM) using FACS ARIA III, and expression of IL-6 and MMP3 in FLS analysed by ultrasensitive qPCR. In addition, the levels of IL-8 and MMP3 proteins were evaluated in co-culture supernatants by ELISA. To investigate the nature of the contact (40min meandering velocity) of individual MDMs on FLS monolayers was evaluated using Delta Vision fluorescence microscopy.

Results: FLS co-cultured with MDM showed an increased mRNA expression of IL-6 at both 24 and 48h, and MMP1 at 48h, compared to FLS cultured alone. This expression pattern was decreased when MDM were pre-treated with Dex and significantly up regulated when MDM were pre-treated with LPS. Consistently, with mRNA expression, IL-6 protein was significantly increased when FLS were co-cultured with MDM (1192±137pg/ml) as compared to FLS cultured alone (445±78pg/ml). This production was significantly augmented when MDM were pre-treated with LPS (1960±34pg/ml) and decreased when MDM were pre-treated with Dex (729±82pg/ml). Similarly, MMP3 protein level was increased when FLS were co-cultured with MDM (256±10pg/ml) as compared to FLS cultured alone (123±1pg/ml). This production was significantly augmented when MDM were pre-treated with LPS (1960±34pg/ml) and decreased when MDM were pre-treated with Dex (164±2pg/ml). These distinct effects of MDM phenotypes on FLS function were associated with different dynamics of their interactions. Our preliminary data reveal that MDM are quiescent on FLS monolayers unless MDM are pre-treated with Dex, which induced a patrolling behaviour. This production was significantly augmented when MDM were pre-treated with LPS (1960±34pg/ml) and decreased when MDM were pre-treated with Dex (164±2pg/ml). These distinct effects of MDM phenotypes on FLS function were associated with different dynamics of their interactions. Our preliminary data reveal that MDM are quiescent on FLS monolayers unless MDM are pre-treated with Dex, which induced a patrolling behaviour.

Conclusion: Macrophages can actively increase or limit activation of FLS, depending on their inflammatory or regulatory phenotypes, respectively. These observations indicate the potential for different subsets of synovial tissue macrophages to drive or resolve synovial inflammation by influencing the stromal compartment.

REFERENCES

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