Selective Targeting Of Monocytes And Macrophages Subsets In The Joint Provides A Potential Therapeutic Approach For Osteoarthritis

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INTRODUCTION: Monocytes and macrophages (MΦs) are key contributors to osteoarthritis (OA) pathogenesis. There is much interest in manipulating MΦs for potential therapeutic advantages. While the elevated presence of MΦs expressing chemokine receptor CCR2 in OA vs. non-OA joint makes the CCR2 a promising target for modulating OA severity, global knock-outs of $CCR2^{+}$MΦs have resulted in contradictory outcomes. This is because different subsets of MΦs commonly express CCR2 but have different homeostatic vs. pro-inflammatory functionality in OA progression, which is not fully understood. To understand the functional roles of different $CCR2^{+}$ MΦs subsets in the joint, we use a tamoxifen (TAM)-inducible $CCR2^{CreER}$:$Rosa26^{Td}$ transgenic mice model to selectively label $CCR2^{+}$cells with tdTomato upon TAM administration. This model enables us to investigate how the population of these labeled cells changes throughout the progression of OA.

METHODS: Mice with experimentally induced OA received TAM chow for two weeks, and the retention of tdTomato after TAM cessation was studied using flow cytometric and immunofluorescence analyses. By altering both the duration of TAM administration (pulse) and the time of tissue harvesting after TAM cessation (chase), we selectively label different populations of MΦs in the OA joint. The animal study was approved by the Animal Care Committee (ARC) review board, University Health Network, Toronto, Ontario, Canada, AUP # 5847.

RESULTS SECTION: Blood monocytes infiltrated the joint during joint inflammation, and some remained CCR2+ while gradually being replaced by unlabeled infiltrating blood monocytes after TAM discontinuation. We identified two distinct tissue macrophage subsets within the joint based on the expression of different MΦs markers and tdTomato. Tissue monocytes were identified as CD64+, MerTK-, CCR2+, TIMD4-, Ly6C hi/low (Figure 1). Monocytes-derived tissue macrophages were identified as CD64+, MerTK+, Ly6C low, CCR2-, TIMD4-, and self-renewing tissue-resident macrophages were identified as CD64+, MerTK+, Ly6C low, CCR2-, TIMD4+ (Figure 2).
CD64: Cluster of Differentiation 64, CCR2: CC chemokine receptor 2, Ly6C: Lymphocyte antigen 6 complex, locus C, MerTK: Mer Tyrosine Kinase, TIMD4: T-cell immunoglobulin and mucin domain-containing protein 4.

DISCUSSION: Our results suggest that a subset of tissue macrophages differentiate from infiltrating monocytes, undergo phenotypic changes, and lose their CCR2 expression and the tdTomato label indicative of their continuing replacement by infiltrating blood monocytes. We identified this population as a pro-inflammatory population within the joint. A different subset of tissue macrophages demonstrated a minimum contribution from blood monocytes, preserving their tdTomato expression as a sign of their self-renewing phenotype. We identified this subset of macrophages as the pro-resolving tissue-resident macrophages. Based on our results, we can label around 50% of the pro-inflammatory MΦs (Figure 1) in the joint, which will increase by increasing the duration of TAM administration up to 80% for 4 weeks pulses. Cessing the TAM for two weeks will only label a few pro-resolving tissue-resident macrophages (Figure 2). We showed that this model system is a valuable tool that allows us to selectively label different populations of MΦs by altering the duration of the TAM pulse and chase.

SIGNIFICANCE/CLINICAL RELEVANCE: This model system can identify and selectively label a subset of pro-inflammatory $CCR2^{+}$ MΦs that populate the joint tissue upon OA injury. Identifying the pro-inflammatory subsets of MΦs in the joint will enable us to label and locally ablate them to diminish OA severity as a potential therapeutic approach for OA.

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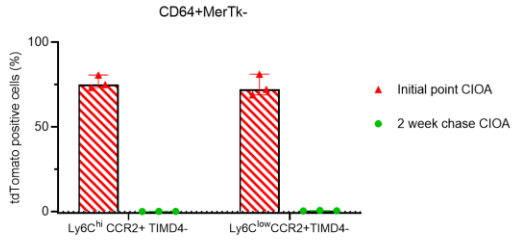

Figure 1

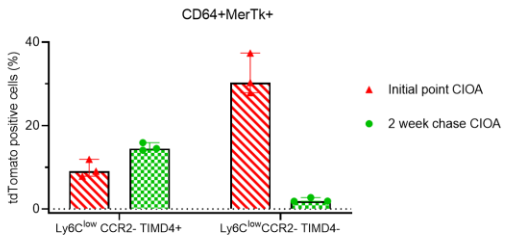

Figure 2

Figure 1: CD64+, MerTK-, CCR2+, TIMD4-, Ly6C hi/low tissue monocytes labeled with tdTomato after two weeks of TAM administration and lost their tdTomato expression two weeks after TAM cessation.
Figure 2: CD64+, MerTK+, Ly6C low, CCR2-, TIMD4+ tissue macrophages minimally labeled with tdTomato after two weeks of TAM administration and retained and increased their tdTomato expression two weeks after TAM cessation, indicative of their proliferative self-renewing phenotype. CD64+, MerTK+, Ly6C low, CCR2-, TIMD4- tissue macrophages labeled with tdTomato after two weeks of TAM administration and lost their tdTomato expression two weeks after TAM cessation, indicative that they originated from blood monocytes.