

Analysis on macrophage subtype alteration in the synovium of rheumatoid arthritis

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INTRODUCTION: Autophagy describes a cellular process of phagocytosis of lysosomes that have fused by cytoplasmic constituent-laden or organelle-laden vesicles and lysosomes¹. By far, the most common function of autophagy is to provide metabolic needs for cells and is regarded as an anti-inflammatory role². Rheumatoid arthritis (RA) is a chronic inflammatory disease with a pathological manifestation of synovial hyperplasia and progressive joint destruction. In our previous study, we found the presence of four types of macrophages in synovial tissues, which are M0 macrophages (CD11b+CD86-CD206-), M1 macrophages (CD11b+CD86+CD206-), M2 macrophages (CD11b+CD86-CD206+), and M1/2 macrophages (CD11b+CD86+CD206+), but autophagy in synovial macrophages of different phenotypes is still unknown. Therefore, we investigated the macrophage subtype alteration and analyzed the relationship between such phenomenon and autophagy.

METHODS: i) Cell extraction: The synovial membrane tissues, which were surgically obtained from active RA (n=5) patients, were cut into fragments, enzymatically and mechanically digested (digestion buffer: Multi Tissue Dissociation Kit 1, Miltenyi Biotec Inc, California, USA) into single cells. The tissue-cell miscible liquids were filtrated by a 70 µm cell strainer (FALCON, New York, USA) to collect the single-cell suspensions. ii) Flow cytometry: Cells were washed twice with cold cell staining buffer (Biolegend, San Diego, CA), incubated with Fc Blocker (Biolegend), and then with fluorochrome-tagged monoclonal antibodies. Surface marker against CD11b (PE, Biolegend) to typify the macrophages, against CD86 (APC, Biolegend) for the M1 phenotype, and against CD206 (FITC, Biolegend) to characterize the M2 phenotype. Upon labeling, cells were suspended in a permeabilization buffer (Fixation/Permeabilization kit, BD Biosciences) and later incubated with biotin-conjugated autophagy-related antibodies—WIP12/LC3/p62 (Biotin, Biolegend), followed by incubating cells with Streptavidin-Pacific blue (ThermoFisher). Flow cytometry measurements were conducted on a BD FACSMelody™ cell sorter (BD Biosciences). Raw data were processed using the software FlowJo (Tree Star, Ashland, OR) iii) Cell culture: Cell suspensions were centrifuged directly at 1500r/min for 5min. Cell layers were left and resuspended with cell culture medium, seeding into 10-cm standard tissue culture plates in 10 mL Roswell Park Memorial Institute 1640 Medium [RPMI 1640; Life Technologies] supplemented with heat-treated 5% fetal bovine serum (FBS, Life Technologies), 1% antibiotic/antimycotic solution (Sigma-Aldrich, St. Louis, MO). The medium was changed three hours later, and only adherent macrophages were left for subsequently 48 hours of culture with or without 20 ng/ml of IFN-γ (R&D system, #285-IF) & 20 ng/ml of LPS (Sigma, #L3137), and 20 ng/ml of interleukin 4 (R&D Systems, #204-IL). Adherent macrophages were washed twice with ice-cold phosphate-buffered saline (PBS, Gibco). Accutase® solution (Sigma-Aldrich, St. Louis, MO), 2 mL, was added to the plate for 10 minutes in the 37°C incubator for cell detachment. Cells were then gently transferred into tubes for flow cytometry test.

RESULTS SECTION: i) Cell extraction: Single cells were successfully isolated from the patient's postoperative tissue (Fig 1, 2). ii) Flow cytometry: We used the strategy for cell sorting to isolate M0 macrophages (CD11b+CD86-CD206-), M1 macrophages (CD11b+CD86+CD206-), M2 macrophages (CD11b+CD86-CD206+), and M1/2 macrophages (CD11b+CD86+CD206+) (Fig 3). In all five cases, the intensity of autophagy reaction was calculated by the Median Fluorescence Intensity (MFI) of autophagy-related intra-cellular protein—WIP12/LC3/p62—in each group cell. The average MFI of M1/2 was stronger than in M0, M1, or M2 (paired t-test) (Fig 4,5,6). iii) Cell culture: Cell culture in-vitro showed a statistically significant result that the number of M0 cells was the lowest in the anti-inflammatory factors culture environment (Fig 7), significant changes in the number of M1 macrophages were seen in the absence of stimulatory factors versus the addition of inflammatory stimulatory factors. However, the quantity of M1/2 double-positive cells decreases as the amount of inflammatory factors in the environment increases, but increases in an anti-inflammatory environment.

DISCUSSION: The mutual transformation of different subtypes of macrophages in various inflammatory environments indicates the mobilization of macrophages and local host responses in RA synovitis. The presence of the unique dual phenotype of M1/M2 and the increased quantity in anti-inflammatory environments also suggest the macrophage transition in synovial inflammation. This is crucial for understanding the pathophysiology of synovitis. However, whether the enhanced autophagic function is associated with subtype switching or anti-inflammatory cellular functions remains to be investigated.

SIGNIFICANCE/CLINICAL RELEVANCE: The present study establishes new and fundamental information regarding the degrees of autophagy in different subtypes of macrophages in RA synovium.

REFERENCES:

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2. Deretic V, et al. Autophagy, 2018;1-9

IMAGES AND TABLES:

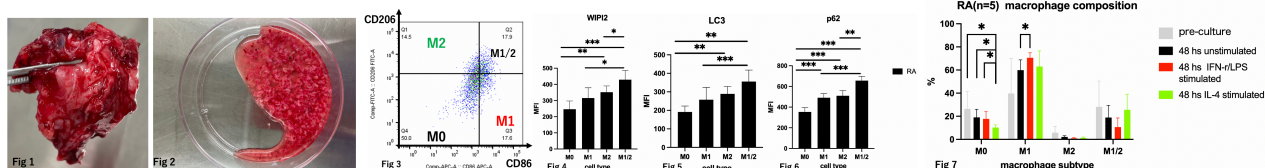


Fig 1,2. Isolation of individual cells from postoperative tissues.

Fig 3. M0 macrophages (CD11b+CD86-CD206-), M1 macrophages (CD11b+CD86+CD206-), M2 macrophages (CD11b+CD86-CD206+), and M1/2 macrophages (CD11b+CD86+CD206+) was got.

Fig 4~6. Comparison of autophagy protein content in different cell subtypes.

Fig 7. Macrophage subtype variation in different inflammatory environments.