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Local fibroblast proliferation but not influx is responsible for synovial hyperplasia in a murine model of rheumatoid arthritis

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ABSTRACT

Synovial fibroblasts play crucial roles in inflammation and joint destruction in rheumatoid arthritis (RA). How they accumulate in the RA joints remains unclear. This study was conducted to discern whether cellular influx from the outside of the joints and local proliferation are responsible for synovial fibroblast accumulation in an animal model of RA. We found that synovial fibroblasts were identified as GFP+ cells using collagen type I alpha 2 (Col1a2)-GFP transgenic reporter mice. Then, bone marrow transplantation and parabiosis techniques were utilized to study the cellular influx. Irradiated wild-type mice were transplanted with bone marrow from Col1a2-GFP mice. Col1a2-GFP and wild-type mice were conjoined for parabiosis. The transplanted mice and the parabionts were subjected to collagen antibody-induced arthritis (CAIA). We found no GFP+ cells in the hyperplastic synovial tissues from the transplanted mice with CAIA and from the wild-type parabionts with CAIA. Furthermore, normal and CAIA synovial tissues from Col1a2-GFP mice and from fluorescent ubiquitination-based cell cycle indicator (Fucci) transgenic mice, in which cells in S/G_2/M phases of the cell cycle express Azami-Green, were studied for Ki67, a cellular proliferation marker, and vimentin, a fibroblast marker, expression. The percentages of Ki67+/GFP+ and Azami-Green+/vimentin+ cells in the CAIA synovial tissues were higher than those in the untreated synovial tissues (34% vs. 0.40% and 19% vs. 0.26%, respectively). These findings indicate that local fibroblast proliferation but not cellular influx is responsible for the synovial hyperplasia in CAIA. Suppression of proliferation of the local synovial fibroblasts should be a promising treatment for RA.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease that presents as polyarthritis with structural joint destruction [1]. Accumulation of fibroblasts and inflammatory cells results in formation of the pannus, which characterizes the pathology of the RA. Synovial fibroblasts play crucial roles in inflammation and joint destruction in RA [2,3] and its animal models including collagen antibody-induced arthritis (CAIA) [4] and collagen-induced arthritis (CIA) [4–6]. However, how the synovial fibroblasts accumulate in the arthritic synovial tissues remains unclear.

Possibly, the hyperplastic pannus might result from increased proliferation of resident synovial fibroblasts [2,7]. In support of this notion, cultured RA synovial fibroblasts proliferate more vigorously and are less sensitive to apoptotic stimuli than cultured synovial fibroblasts derived from the osteoarthritis joints [8,9]. However,

Abbreviations: RA, rheumatoid arthritis; CAIA, collagen antibody-induced arthritis; CIA, collagen-induced arthritis; Col1a2, collagen type I alpha 2; Col2, type II collagen; MSC, mesenchymal stem cell.

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prevalence of proliferating synovial fibroblasts estimated with the Ki67, proliferating cell nuclear antigen (PCNA) and c-myc staining varied among the studies of the RA synovial tissues [10–14].

Alternatively, the synovial fibroblasts might accumulate via influx of non-resident cells from the outside of the joints. Recent studies have reported that bone marrow-derived mesenchymal cells, circulating fibrocytes, and synovial fibroblasts can migrate from the outside of the joints in animal models of RA [15–19]. However, it still remains unclear whether and to what extent the local cellular proliferation and the influx of non-resident cells contribute to the synovial fibroblast accumulation in RA and its animal models.

Ki67, a common cellular proliferation marker, is expressed in G_1, S, G_2, and M phases but not in a quiescent (G_0) phase of the cell cycle. It is expressed not only by proliferating cells but by cells being arrested in the G_1 phase and entering the G_0 phase from the G_1 phase [20,21]. In fluorescent ubiquitination-based cell cycle indicator (Fucci) transgenic reporter mice, every cell emits either green fluorescence (Azami-Green) or red fluorescence (Kusabira-Orange) [22,23]. Azami-Green is positive in the S, G_2, M phases while Kusabira-Orange is positive in the G_1 and G_0 phases. Although Azami-Green+ cells in these mice do not include the proliferating cells entering the S phase from the G_1 phase, studies of the fluorescence in the Fucci mice and Ki67 expression should collaborate to demonstrate the cellular proliferation precisely.

The present study was conducted to the contribution of the local cellular proliferation and the cellular influx to the synovial hyperplasia. To study the influx of non-resident cells into the arthritic joints, we utilized bone marrow transplantation and parabiosis techniques. In our previous reports, these techniques have revealed the presence or absence of the cellular influx in multiple disease models including skin fibrosis, pulmonary fibrosis, and liver fibrosis [24–26]. They should also be of use for studying the origin of the synovial fibroblasts in arthritis models.

2. Material and methods

2.1. Mice

Collagen type I alpha 2 (Col1a2)-GFP transgenic mice on a C57BL/6 background were established previously [26]. FucciG_1-#639/FucciS/G_2/M-#474 double transgenic mice on a C57BL/6 background were gifts from A. Miyawaki (RIKEN BioResource Center, Ibaraki, Japan) [22]. Wild-type C57BL/6 (CD45.2+) and B6.SJL (CD45.1+) mice were purchased from Charles River Japan (Kanagawa, Japan) and the Jackson Laboratory (Bar Harbor, ME) respectively. Experiments were performed on 6- to 10-week-old male and female mice. All procedures in the animal experiments were approved by the Institutional Animal Care and Use Committee of TMDU.

2.2. Induction of arthritis

CAIA was induced by the administration of 5 mg of Arthrogen-CIA antibodies (Chondrex, Redmond, WA) and lipopolysaccharide (serotype 0111:B4) according to the manufacturer's instruction.

2.3. Bone marrow transplantation

Wild-type C57BL/6 or B6.SJL mice was administered a single 10-Gy dose of whole body irradiation. After 6 h, 5 million unfractionated bone marrow cells from Col1a2-GFP mice were injected intravenously. Bone marrow reconstitution was confirmed with the chimerism of the peripheral blood from the recipients using either real-time PCR analysis quantifying a GFP transgene normalized to an internal control, glyceraldehyde-3-phosphate dehydrogenase gene as described previously [24] or flow cytometric analysis with PE anti-CD45.1 (clone A20, 12-0453; eBioscience, San Diego, CA) and APC anti-CD45.2 (clone 104, 17-0454; eBioscience) antibodies.

2.4. Parabiosis

Pairs of age matched male Col1a2-GFP mice and wild-type littermates were surgically joined for parabiosis. Cross-circulation was confirmed with the chimerism using the pairs of peripheral blood as described above.

2.5. Immunostaining and histological analysis

Frozen sections of murine knee joints were studied histologically using a confocal microscope. Five random fields of view were observed for each section. Cell counts were assessed in 1 section per joint (1 joint per mouse). The experiments using bone marrow transplantation and parabiosis techniques were performed on 2 sections per joint (1 joint per mouse). Detailed protocol and materials used are described in Supplementary materials and methods.

2.6. Flow cytometric analysis

The peripheral blood and disaggregated knee joint tissues were harvested from mice with CAIA (1 joint per mouse). Detailed protocol and materials used are described in Supplementary materials and methods.

2.7. Statistical analysis

The number of cells was analyzed statistically using unpaired t-test (two-tailed) for comparisons between 2 groups. One-way analysis of variance was used with Bonferroni correction for comparisons between 3 groups.

3. Results

3.1. Accumulation of synovial fibroblasts as GFP+ cells in Col1a2-GFP mice during CAIA

The fibroblasts in Col1a2-GFP transgenic mice that express GFP in collagen type I-producing cells have been identified in other studies as GFP+ cells in the models of wound healing, liver fibrosis, pulmonary fibrosis, and adipose tissue fibrosis [24–28]. To study if the synovial fibroblasts can be identified as GFP+ cells similarly in the same reporter mice, they were subjected to administration of anti-type II collagen (Col2) antibodies. As was reported earlier [29], after the antibody injection (day 0), arthritis peaked day 5–8, persisted until at least day 10, and gradually decreased until day 20. When synovial thickening was maximal (day 10), the joints were collected for histological studies with confocal microscopy.

The untreated Col1a2-GFP mice had relatively few GFP+ cells in the synovial tissues, while the arthritic Col1a2-GFP mice demonstrated frequent GFP+ cells (Fig. 1A). Next, we examined the GFP+ cells for expression of cell-type specific antigens. All GFP+ cells expressed the common fibroblast marker, vimentin (Fig. 1B). They also expressed another fibroblast marker, Hsp47 [30] (Fig. 1C). Thus, the GFP+ cells in the reporter mice were all synovial fibroblasts. In addition, the number of GFP+ cells reached a maximum and was as abundant as that of leukocytes, CD45+ cells, when the arthritis peaked (day 10) (Fig. 1D). Thus, the synovial fibroblasts as well as the leukocytes accumulated in the hyperplastic synovial tissues in CAIA.