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Functional folate receptor beta-expressing macrophages in osteoarthritis synovium and their M1/M2 expression profiles

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Objective: The distribution of folate receptor (FR)-β+ macrophages and their M1/M2 expression profiles were examined in osteoarthritis (OA) synovial tissues, and compared to those in rheumatoid arthritis (RA) synovial tissues and CD163+ macrophages in both OA and RA synovial tissues.

Method: The phenotypes and fluorescein isothiocyanate (FITC)–folate uptake of FR-β+ synovial macrophages were analysed by flow cytometry. The distribution of FR-β+ macrophages in OA and RA synovial tissues was examined by immunofluorescent microscopy. Tumour necrosis factor (TNF)-α, inducible nitric oxide synthase (iNOS), interleukin (IL)-10, and transforming growth factor (TGF)-β expression in FR-β+ macrophages was detected by double-immunostaining in both OA and RA synovial tissues.

Results: FR-β+ macrophages were predominantly present in the synovial lining layer in OA patients. The proportion of CD163–FR-β+ cells in synovial mononuclear cells (MNCs) was increased in OA compared to RA synovial tissues. FR-β^{high} macrophages from OA synovial tissues represented the majority of folic acid-binding cells. Although FR-β+ or CD163+ macrophages in the synovial tissues of OA and RA patients expressed a mixed pattern of M1 and M2 macrophage markers, there were more M2 markers expressing synovial macrophages in OA than in RA patients.

Conclusions: The distribution and M1/M2 expression profiles of FR-β+ synovial macrophages were different between OA and RA synovial tissues. Thus, the findings underscore that the M1/M2 paradigm using surface markers FR-β and CD163 is an oversimplification of macrophage subsets. Functional FR-β present on OA synovial macrophages provides a potential tool for the diagnosis and treatment of OA.

Macrophages are heterogeneous with regard to their morphology, location, and function in normal and diseased tissues (1, 2). In osteoarthritis (OA) synovial tissues, infiltrated macrophages are predominantly present in the synovial lining layer (3–5). These macrophages are thought to be the pivotal cell type in the synovial tissues that mediate osteophyte formation and other OA-related pathologies, such as fibrosis, during experimental OA (6–8). The macrophages at the site of inflammation display immune polarization and promote inflammation; they are referred to as classically activated macrophages (M1 macrophages). Alternatively, activated macrophages (M2 macrophages) (9, 10) regulate inflammation, along the T-helper (Th)1 and Th2 paradigm. It has been reported that the expression of several macrophage surface markers reflects M1 or M2 macrophage function. These reports were mainly based on in vitro data, which were collected using murine and human macrophages. However, little is known about whether these differently polarized macrophages express unique surface markers in the lesions of various diseases including inflammatory synovitis.

Folate receptors (FRs) are a family of glycosylphosphatidylinositol-anchored glycoproteins with a high affinity for folic acid and 5-methyltetrahydrofolate (11). Because of overexpression of FR-α on cancer cells and FR-β on activated macrophages, significant progress has been made with regard to FR-targeted drugs for the diagnosis and therapy of cancers and inflammatory/autoimmune diseases (12). We previously found that FR-β+ macrophages heavily infiltrated rheumatoid arthritis (RA) synovial tissues (13, 14). In addition, it has been reported that FR-β+ macrophages produce proinflammatory cytokines (15). Thus, it has been considered that FR-β+ macrophages act as M1 macrophages in the pathogenesis of rheumatoid synovitis. In fact, we demonstrated previously that the depletion of FR-β+ macrophages, by an immunotoxin to FR-β, reduced the activity of RA synovitis that was implanted in severe combined immunodeficiency (SCID) mice (16). Intriguingly, we noted that most of these FR-β+ macrophages

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co-expressed CD163 (13), a marker of M2 macrophages (17, 18). More recently, we and others reported that some tumour-associated macrophages that are characterized as M2 macrophages expressed FR-β in the stroma of several cancers (19, 20). Thus, it remains controversial whether FR-β expression reflects M1 function in the lesions of various diseases. FolateScan, which detects folate receptors, is being used to identify up-regulation of FRs in the joints, and RA patients have more FR expression in their joints than non-arthritic patients (21). Of interest, joint uptake was also seen in OA patients. However, it is unclear whether macrophages in OA synovial tissues express FR-β and whether these macrophages express M1 or M2 markers at inflamed sites. Because most macrophages in normal human tissues express little or no FR-β, characterization of FR-β+ macrophages in OA synovial tissues may be beneficial in the treatment of OA through drugs targeting FR-β.

To better characterize the inflammatory milieu that is related to macrophages in OA synovial tissues, this study was undertaken to investigate FR-β expression on OA synovial macrophages. Moreover, the presence of FR-β+ macrophages in OA synovial tissues led us to investigate whether they act as either M1 or M2 macrophages.

Materials and methods

Tissue samples

Tissue samples were taken from the synovium of 15 OA patients [four males, 11 females; age 73.5 ± 8.7 years (mean ± sd); disease duration 13.0 ± 3.8 years (mean ± sd)] and 12 RA patients [two males, 10 females; age 62.9 ± 10.6 years (mean ± sd); disease duration 17.9 ± 12.1 years (mean ± sd)] who underwent total knee replacement surgery. The disease diagnosis was performed using the American Rheumatism Association criteria for OA (22) and RA (23). All patients with OA were treated with non-steroidal anti-inflammatory drugs (NSAIDs), while all of the patients with RA were treated with NSAIDs and/or less than 5 mg of prednisolone. In addition, eight patients were treated with gold salts, bucillamine, sulfasalazine, methotrexate (MTX), tacrolimus, remicade, or a combination of these drugs. Informed consent was obtained from all of the donors in accordance with the requirements of the Human Investigation Committee of Kagoshima University.

Isolation of synovial mononuclear cells (MNCs) and flow cytometry analysis

MNCs were isolated from the OA and RA synovium for phenotypic analysis and cell sorting, as described previously (24). In brief, synovial tissues were digested with type V collagenase and cell suspensions were isolated on a Ficoll/Hypaque gradient, incubated in plastic plates. These adherent cells were used for the flow cytometric analysis and sorting. After blocking non-specific binding, the cells were stained with Alexa Fluor 488-conjugated anti-CD14 (BioLegend, San Diego, CA, USA), Alexa Fluor 488-conjugated anti-CD163 (14), biotinylated anti-FR-β (14), or isotype-matched control monoclonal antibodies (mAbs). The biotinylated mAbs were detected after incubation with allophycocyanin (APC)-conjugated streptavidin (BD Biosciences, San Jose, CA, USA). 7-Amino-actinomycin D (7-AAD; eBioscience, San Diego, CA, USA) was used to identify living cells. The stained cells were analysed and sorted in the gate including macrophages by fluorescence-activated cell sorting (FACS) using FACSAria (Becton Dickinson, Franklin Lakes, NJ, USA). The sorting purity was more than 90% for each experiment.

FACS-sorted cells were cytospun to glass slides and fixed with acetone for CD68 staining. The endogenous peroxidase was blocked by 0.6% H_2O_2 at room temperature for 10 min. After washing with phosphate-buffered saline (PBS), the cells were treated with the Dako Cytomation Biotin Blocking System (Dako, Carpinteria, CA, USA) according to the manufacturer's instructions. The cells were then incubated with PBS containing 1% bovine serum albumin (BSA) and 10% AB serum for 30 min at 37°C. The cells were then treated with biotinylated anti-CD68 (BioLegend) or isotype-matched control mAb for 1 h at 37°C. After washing, the cells were incubated with horseradish peroxidase-conjugated streptavidin (ZYMED, South San Francisco, CA, USA) for 30 min at 37°C. Visualization was performed using NovaRED (Vector Laboratories, Burlingame, CA, USA) and the cell nuclei were counterstained with haematoxylin.

Phagocytosis assay

A phagocytosis assay was performed as described previously (25). In brief, 1×10^5 cells were incubated with fluorescein isothiocyanate (FITC)-dextran (MW 40 000; Molecular Probes, Eugene, OR, USA) in 1 mg/mL Iscove's Modified Dulbecco's Medium (IMDM) with 10% foetal bovine serum (FBS) for 45 min at 37°C. After washing extensively with cold PBS containing 2 mM ethylenediaminetetraacetic acid (EDTA), 0.5% BSA, and 0.1% NaN_3, the cells were stained with biotinylated anti-FR-β mAb and anti-CD163 mAb, or isotype-matched control mAb, and then mixed with streptavidin-Alexa Fluor 647 (or APC-Cy7) (Invitrogen, Tokyo, Japan) for an additional 30 min at 4°C to detect biotinylated mAb. FITC-dextran uptake was analysed by FACS and expressed as the mean fluorescence intensity (MFI) of FITC-dextran staining.

Folate uptake assay

FITC-folate uptake was measured as described previously (14). In brief, synovial MNCs were washed with acid buffer to remove the cell surface-bound folic acid. After

blocking non-specific binding with Hanks' balanced salt solution (HBSS) containing 10% dialysed human AB serum, the cells that contained 1% dialysed foetal calf serum (FCS) were incubated with biotinylated anti-CD163, biotinylated anti-FR-β, or biotinylated isotype matched control mAbs for 30 min at 4°C. After washing with HBSS, the cells were incubated with FITC-folate (500 ng/mL) (a gift from Prof. Low, Purdue University) and APC-streptavidin for 45 min at room temperature. As a negative control, 1000-fold excess amount of folic acid was added 5 min prior to the addition of FITC-folate. The amount of FITC-folate uptake was analysed by FACS and expressed as the MFI.

Immunofluorescent analysis of synovial tissues

Acetone-fixed synovial frozen sections were blocked with PBS containing 1% BSA and 10% AB serum for 30 min at 37°C, followed by incubation with Alexa Fluor 488-conjugated anti-CD163 and biotinylated anti-FR-β or isotype-matched control mAbs for 1 h at 37°C. The sections were incubated with Rhodamine 600 Avidin D (Vector Laboratories) for an additional 1 h at 37°C, washed extensively in PBS, and counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (2 ng/μL). The stained cells were imaged using a fluorescent microscope (BZ-8000, Keyence, Osaka, Japan) and counted for each synovial layer in 10 randomly selected high-power (×400) fields.

Immunohistochemical analysis of synovial tissues

Two-colour immmunohistochemical staining was performed as described previously (26). In brief, 5-μm tissue sections were fixed with cold acetone and treated with 0.6% H_2O_2 for 10 min to quench the endogenous peroxidase. After further blocking non-specific binding with the Protein Block Serum-Free solution (DAKO), the sections were stained with one of the following first primary antibodies at 5 μg/mL: rabbit anti-transforming growth factor (TGF)-β1 Ab (sc-146, Santa Cruz Biotechnology, Santa Cruz, CA, USA); rabbit anti-inducible nitric oxide synthase (iNOS) Ab (sc-651, Santa Cruz Biotechnology); goat anti-human tumour necrosis factor (TNF)-α Ab (AF-410-NA, R&D Systems, Minneapolis, MN, USA). Each first antibody was visualized with a MAX-PO secondary Ab (Nichirei Co. Ltd, Tokyo, Japan) and a DAB staining kit (DAKO). To further stain with each second primary antibody, the sections were incubated with 0.1 M glycine-HCl (pH 2.7) for 1 min to remove residual antibody and washed extensively with TBST (20 mM Tris–HCl, pH 7.4, 0.15 M NaCl, 0.1% Tween-20). These sections were then sequentially incubated with 1 μg/mL of the following mAbs: mouse anti-human CD163 mAb (14) or mouse anti-human FR-β mAb (14) and an alkaline-phosphatase conjugated MAX-PO secondary Ab (Nichirei). The staining for each second primary Ab was visualized using the Vector Blue kit (Vector Laboratories). Stained cells were imaged using a microscope equipped with a digital

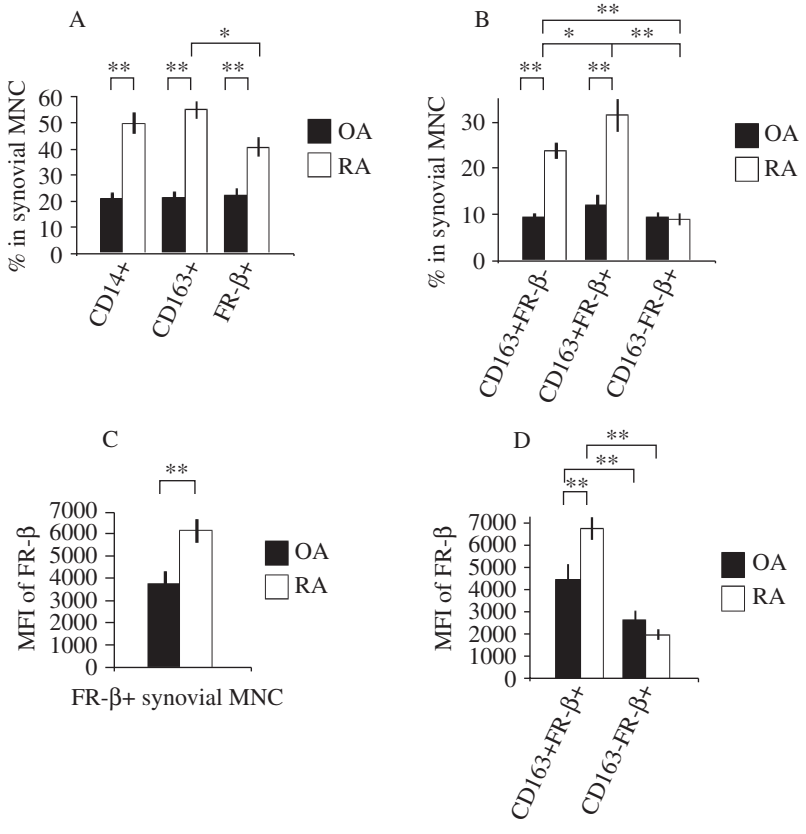

Figure 1. Phenotypic characterization of FR-β+ macrophages in OA and RA synovial mononuclear cells. Synovial tissues from patients with OA (n = 15) or RA (n = 12) were digested with type V collagenase. Single adherent MNC suspensions were prepared by Ficoll/Hypaque gradient centrifugation followed by plastic plate adherence. These MNCs were stained with fluorochrome-conjugated mAbs against CD14, CD163, and/or FR-β. The relative numbers of individual subsets in gated MNCs were calculated as a percentage of total synovial MNCs, and the expression levels of FR-β in each subset were measured by the mean fluorescence intensity (MFI) of anti-FR-β mAb staining. (A) The relative number of CD14+, CD163+, and FR-β+ cells in the synovial MNCs from OA and RA patients. (B) The relative number of CD163+FR-β−,CD163+ FR-β+, and CD163–FR-β+ cells in the synovial MNCs from OA and RA patients. (C) The expression levels (MFI) of FR-β in FR-β cells of the synovial macrophages from OA and RA patients. (D) The expression levels (MFI) of FR-β in the subsets of CD163+FR-β+ and CD163–FR-β+ synovial MNCs from OA and RA patients. All data are given as mean ± SEM. Mann–Whitney U-test, *p < 0.05 and **p < 0.01 between the two groups.

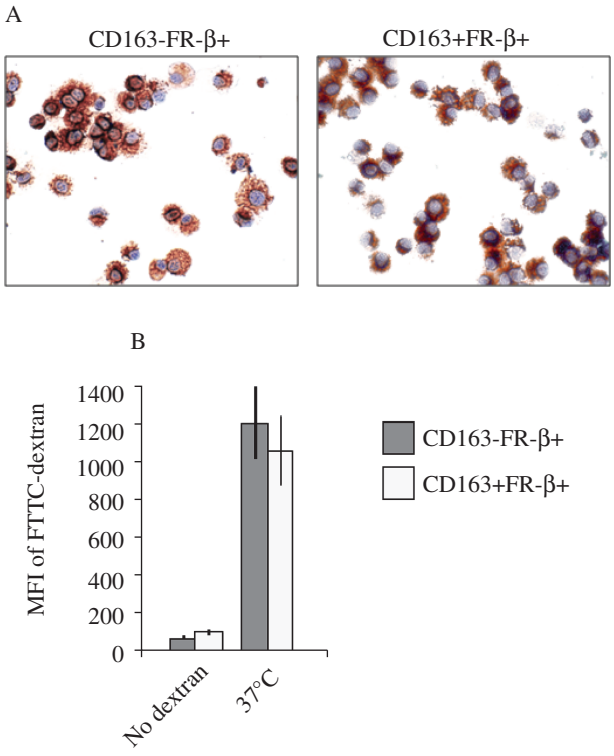

Figure 2. Macrophage phenotype and phagocytosis of CD163-FR-β+ cells in OA synovial MNCs were prepared from the synovial tissues of OA patients, stained with the fluorochrome-conjugated mAbs against CD163 and FR-β, and FACS-sorted. (A) Immunocytochemical staining revealed that CD68, a pan-macrophage marker, was expressed by CD163–FR-β+ and CD163+FR-β+ synovial MNCs. Red: CD68+ cells. Blue: nuclear counterstain (haematoxylin). Depicted are synovial MNCs from three OA patients. (B) Sorted CD163–FR-β+ and CD163+FR-β+ synovial MNCs and were incubated with FITC-dextran or medium alone for 1 h at 37°C, and FITC-dextran uptake was analysed by FACS. Both subsets of synovial MNCs phagocytosed FITC-labelled dextran equally well. Data are given as mean ± SEM for the MFI of FITC-dextran. n = 4 in each group.

sight CCD camera (DS-Fi1, Nikon, Tokyo, Japan). Semi-quantitative analyses were carried out using a computer-aided image analyser (NIS-Elements, Nikon). The red/blue-stained cells were counted in the lining and sublining layers of 10 randomly selected high-power (×400) fields from each section. A colour threshold mask for immunostaining was defined to detect red and blue colours by sampling. This threshold was applied to all of the samples.

Statistical analysis

All data are presented as mean ± SEM. The non-parametric Mann–Whitney U-test was used to test statistical differences between the two groups. $p < 0.05$ was considered to be statistically significant.

Results

Expression of FR-β on OA synovial macrophages

Synovial MNCs of the patients with OA or RA were stained with an mAb against CD14, CD163, or FR-β, and analysed by FACS. The relative numbers of CD14+, CD163+, and FR-β+ cells in total synovial MNCs were significantly lower in OA patients than in RA patients (Figure 1A). Two-colour cell suspension immunofluorescence staining identified three subsets of synovial MNCs based on the expression of CD163 and FR-β: CD163+FR-β−, CD163+FR-β+, and CD163–FR-β+ cells (Figure 1B). Equal numbers of the three subsets were seen in OA patients. By contrast, CD163+FR-β− and CD163+FR-β+ cells outnumbered CD163–FR-β+ cells in RA patients. Moreover, FR-β+ synovial MNCs from RA patients expressed significantly higher levels of FR-β, as measured by MFI of anti-FR-β

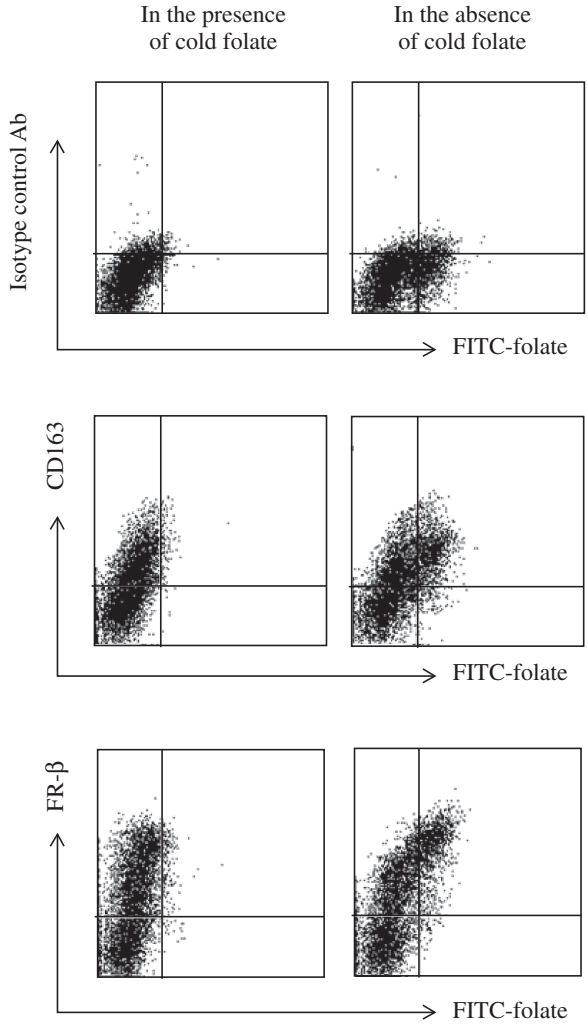

Figure 3. FITC-folate uptake of OA synovial MNCs with high expression of FR-β. Synovial MNCs from OA patients were incubated with a biotinylated mAb against CD163, FR-β or with an isotype control mAb for 30 min at 4°C followed by an additional 30 min incubation with APC-streptavidin. These cells were then incubated with FITC-conjugated folate (500 ng/mL) at room temperature for 45 min in the presence or absence of 1000-fold excess amounts of folate. Most FR-β^{high} and a few FR-βl^{ow} macrophages bound FITC-folate (lower panel). By contrast, the CD163^{high} subset was not necessarily able to uptake the folate (middle panel). There was no binding of FITC-folate onto synovial MNCs in the presence of excess amounts of folate. This experiment was repeated using synovial MNCs from four OA patients.

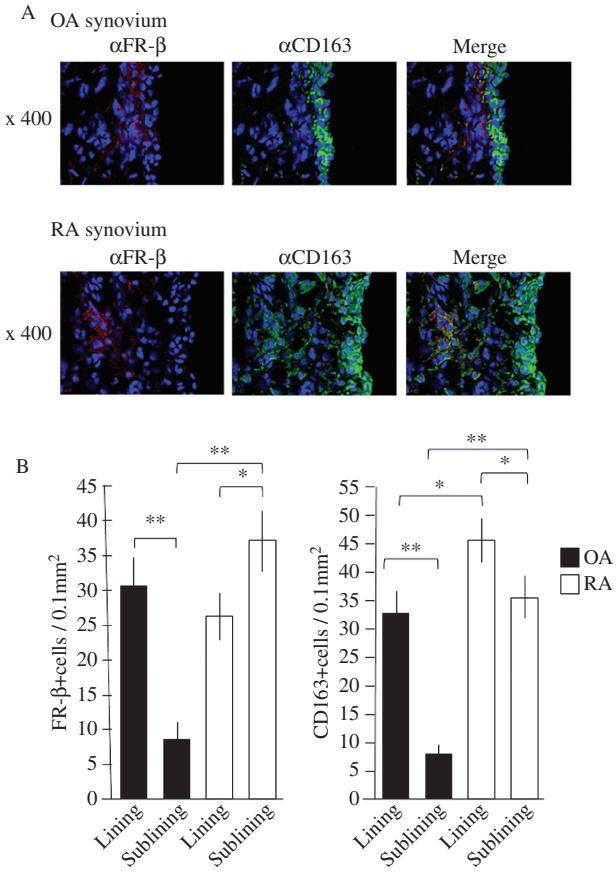

Figure 4. Distribution of FR-β+ macrophages in OA and RA synovial tissues. Frozen synovial sections from patients with OA or RA were stained with mAbs against FR-β (rhodamine, red) and CD163 (Alexa fluor 488, green) followed by nuclear counterstaining with DAPI (blue). FR-β+ cells and CD163+ cells in each layer were counted and quantified as positive cells/0.1 mm^2. (A) Representative immunostaining images show that most FR-β+ macrophages in the synovial tissues from OA patients were located throughout the lining layer. By contrast, FR-β+ macrophages were mainly distributed throughout the sublining layer of the synovial tissues from RA patients. Original magnification: ×400. (B) Quantification of FR-β+ and CD163+ macrophages in each synovial layer of OA and RA patients. Data are given as mean ± SEM from 12 patients in each group. Mann–Whitney U-test, *$p < 0.05$ and **$p < 0.01$ between the two groups.

mAb staining, than those from OA patients (Figure 1C). In particular, FR-β expression levels in the CD163+FR-β+ cells from either OA or RA patients were significantly higher than those in CD163–FR-β+ cells. Additionally, the expression levels of FR-β in CD163+FR-β+ cells from OA patients were significantly lower than that those in cells from RA patients (Figure 1D).

To characterize whether CD163–FR-β+ synovial MNCs from OA patients are macrophages, FACS-sorted synovial CD163–FR-β+ cells were analysed for the expression of CD68, a pan-macrophage marker, and their ability to phagocytose FITC-dextran as compared to CD163+FR-β+ cells. Immunocytochemical staining showed that, similar to CD163+FR-β+ cells, most CD163–FR-β+ cells expressed CD68 (Figure 2A). Functionally, CD163–FR-β+ cells phagocytosed the dextran as well as CD163+FR-β+ cells (Figure 2B).

Folate uptake of FR-β+ macrophages in OA synovial MNCs

FACS analysis of OA synovial MNCs identified two subsets of FR-β+ macrophages: FR-β^{high} and FR-β^{low} cells (Figure 3). In folate uptake assays, folate-uptake macrophages were enriched in the FR-β^{high} subset although a few FR-β^{low} macrophages also took up the folate. By contrast, the $CD163^{high}$ subset was not necessarily able to uptake the folate. Also shown in Figure 3, there was no folate uptake in either FR-β^{high} or CD163+ macrophages in the presence of excess amounts of unlabelled folic acid.

Distribution of FR-β+ macrophages and their polarization in OA and RA synovial tissues

Using two-colour tissue immunofluorescence staining, we examined the location of FR-β+ macrophages in the inflamed synovial tissues of OA patients compared to RA patients. Consistent with our previous report (14), FR-β+ macrophages were distributed predominantly in the sublining layer of RA synovial tissues (Figure 4). By contrast, these macrophages were distributed predominantly in the lining layer of OA synovial tissues. Also shown in Figure 4, CD163+ cells demonstrated similar staining patterns in OA synovial tissues.

Macrophages preferentially produce TNF-α and iNOS after Th1 cytokine stimulation whereas they produce interleukin (IL)-10 and TGF-β after Th2 cytokine stimulation (9, 10). Thus, TNF-α and iNOS are considered M1 macrophage markers, while IL-10 and TGF-β are considered M2 macrophage markers. CD163 is also used as a human M2 macrophage marker. To compare the expression of M1 and/or M2 markers in FR-β+ and CD163+ macrophages in OA synovial tissues, we stained synovial FR-β+ or CD163+ macrophages for M1 (TNF-α and iNOS) (Figure 5) and M2 markers (IL-10 and TGF-β) (Figure 6). The comparison was also made between OA and RA patients.

The proportions of TNF-α+ cells in FR-β+ or CD163+ macrophages of both synovial layers were significantly lower in OA than in RA patients (Figure 5). In OA synovial tissues, more TNF-α+ cells in FR-β+ or CD163+ macrophages were found in the lining layer compared to the sublining layer. By contrast, the proportions of iNOS+ cells in FR-β+ or CD163+ macrophages of both layers were similar between OA and RA patients. In both OA and RA patients, more iNOS+ cells in FR-β+ or CD163+ macrophages were found in the synovial lining layer than in the sublining layer. In both OA and RA patients, there were more IL-10+ cells and TGF-β+ cells in the FR-β+ or CD163+ macrophages of the lining layer than the sublining layer (Figure 6). Apart from more TGF-β cells in FR-β+ macrophages of the lining layer in OA patients (Figure 6B), no significant difference in the expression of either IL-10 or TGF-β was found between OA and RA patients.

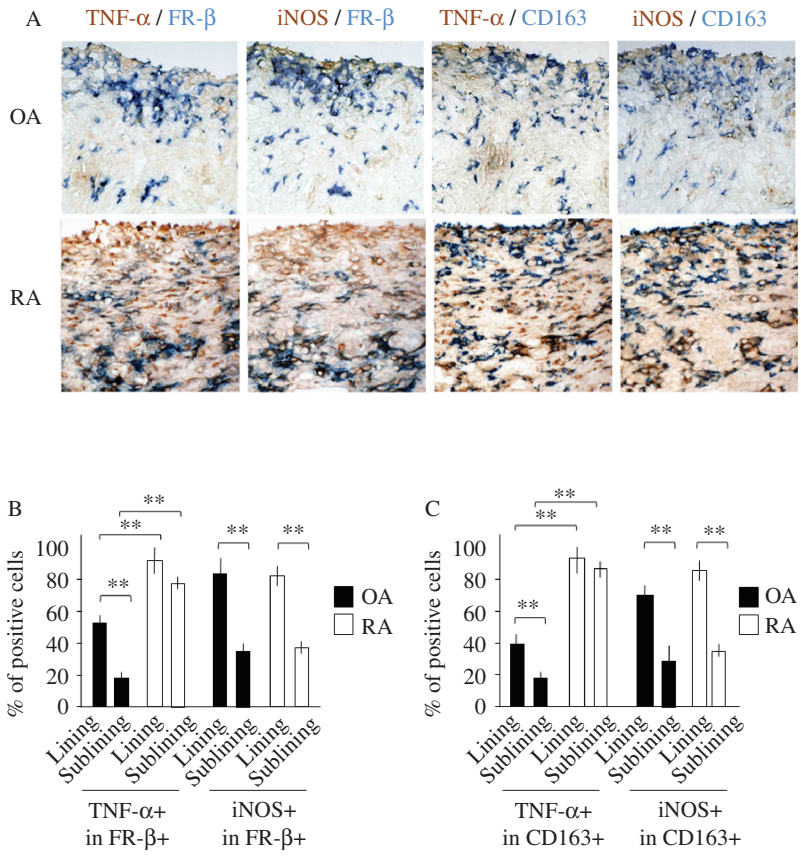

Figure 5. Expression profiles of M1 markers in FR-β+ and CD163+ macrophages in OA and RA synovial tissues. Frozen sections from patients with OA or RA were stained with the combination of an anti-FR-β mAb (or an anti-CD163 mAb) (blue) with either an anti-TNF-α or iNOS antibody (brown). TNF-α- or iNOS-expressing cells in FR-β+ macrophages in each synovial layer were counted and expressed as the percentage in FR-β+ or CD163+ macrophages. (A) Representative immunostaining images depict the expression of TNF-α or iNOS in FR-β+ and CD163+ macrophages. Original magnification: ×400. (B, C) Quantification of TNF-α- or iNOS-expressing cells in (B) FR-β+ macrophages and (C) CD163+ macrophages in each synovial layer of OA and RA patients. Data are given as mean ± SEM from 12 patients in each group. Mann–Whitney U-test, **p < 0.01 between the two groups.

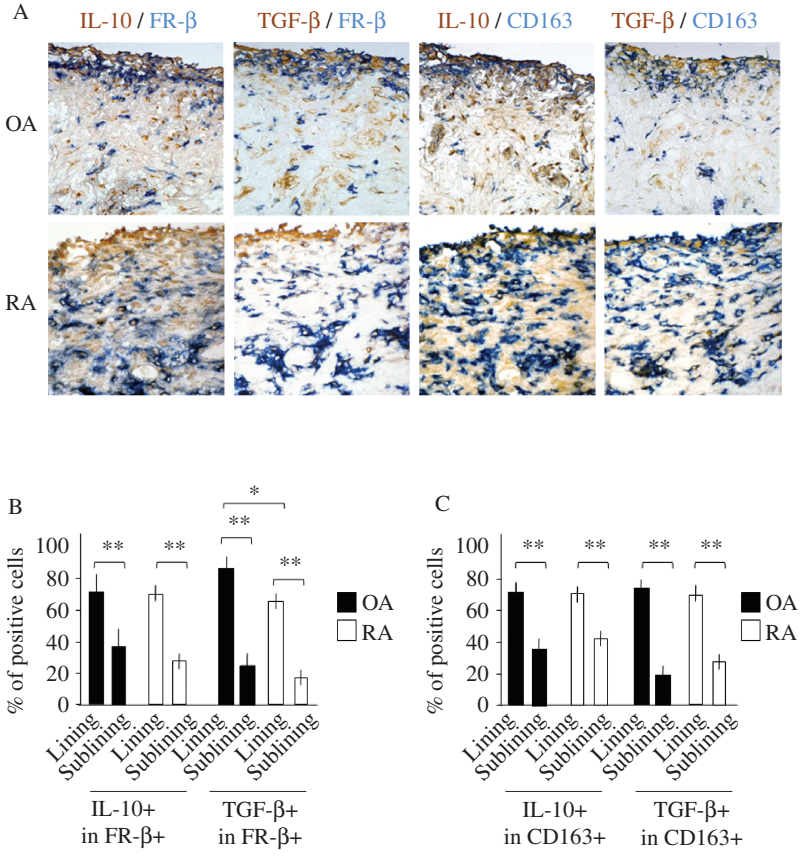

Figure 6. Expression profiles of M2 markers in FR-β+ and CD163+ macrophages in OA and RA synovial tissues. Frozen sections from patients with OA or RA were stained with the combination of an anti-FR-β mAb (or an anti-CD163 mAb) (blue) with either an anti-IL-10 or TGF-β antibody (brown). IL-10- or TGF-β-expressing cells in FR-β+ macrophages in each synovial layer were counted and expressed as the percentage in FR-β+ or CD163+ macrophages. (A) Representative immunostaining depicts the expression of IL-10 or TGF-β in FR-β+ and CD163+ macrophages. Original magnification: ×400. (B, C) Quantification of IL-10- or TGF-β-expression in (B) FR-β+ macrophages and (C) CD163+ macrophages in each synovial layer of OA and RA patients. Data are given as mean ± SEM from 12 patients in each group. Mann–Whitney U-test, **p < 0.01 between the two groups.

Discussion

We have shown that synovial MNCs in OA patients express FR-β and the proportion of CD163–FR-β+ cells in synovial MNCs increased significantly in OA patients compared to RA patients. CD163, which acts as a receptor for haemoglobin/haptoglobin complexes, is exclusively expressed in monocytes and macrophages (27). Glucocorticoid has been shown to up-regulate the expression of CD163 (28, 29). As all RA patients in our study received no, or less than 5 mg, prednisolone daily, it is unlikely that steroid treatment can completely account for the increased proportion of CD163+FR-β+ cells in RA synovial MNCs. Additionally, proinflammatory cytokines such as interferon (IFN)-γ and TNF-α down-regulate CD163 whereas IL-6 and other anti-inflammatory cytokines up-regulate CD163 (30). Because the local production of these cytokines in RA patients is quantitatively and qualitatively different from that in OA patients (31–33), the combination of cytokines may in part determine the expression patterns of FR-β and CD163.

We found that either FR-β+ or CD163+ macrophages in the lining layer of OA synovial tissues preferentially expressed M2 markers as opposed to in RA synovial tissues. In both OA and RA patients, M1 and M2 markers were highly expressed on FR-β+ or CD163+ macrophages in the lining layer as compared to those in the synovial sublining layer, suggesting that lining layer macrophages were more activated regardless of M1 or M2 type. The expression profiles of FR-β and CD163 in the macrophages of OA synovial tissues are consistent with the expression of CD68 as reported previously (34–36). Mouse FR-β+ peritoneal macrophages have been reported to produce TNF-α and reactive oxygen species when stimulated with thiogycolate, zymosan, or bacteria (15). By contrast, a subset of tumour-associated macrophages, considered M2 macrophages, also expressed FR-β (19, 20). FR-β can be induced in CD14+ monocytes when stimulated with cytokines such as macrophage colony-stimulating factor (M-CSF), granulocyte–macrophage colony-stimulating factor (GM-CSF), IL-6, and IL-10 (20). It has not been well documented how FR-β is regulated in inflamed tissues. In most cases, the patterns of gene expression of macrophages in response to various stimuli or to their tissue environment are heterogeneous and do not precisely fit either M1 or M2 patterns (37). For example, CD163 macrophages, although previously classified as M2 macrophages, also identify M1 macrophages in psoriasis skin (38). Taken together with this study, caution should be taken when FR-β and CD163 are used to phenotype M1 and M2 macrophages.

The fact that anti-TNF therapy targets synovial macrophages has led to research for more promising macrophage-targeting drugs to treat RA patients (39). We found that a subset of synovial macrophages from OA patients expressed higher levels of FR-β and took up folate more efficiently. Although we previously showed that FR-β mediated the accumulation of MTX, an anti-RA drug, in synovial macrophages (14), MTX had a limited effect on experimental OA (40). Our findings that OA synovial MNCs expressed low levels of FR-β, and only FR-β^{high} macrophages took up folate, provide a partial explanation for the different therapeutical response to MTX in OA and RA. Encouragingly, several recently discovered drugs that have a high affinity for FR-β promisingly suppressed arthritis and systemic lupus erythematosus in animal models (41–43).

Our findings have potential significance for clinical applications. First, although proinflammatory cytokines and other mediators are crucial for the development of synovial inflammation and for the activation of chondrocytes in OA disease (44, 45), low-grade inflammation in OA joints makes it difficult to assess disease activity using these mediators as biomarkers. FolateScan, which detects the FR-mediated uptake of a folate-derived probe, has successfully monitored disease severity, even light to mild inflammation in RA patients (21). Thus, our finding that FR-β is expressed by OA synovial macrophages will promote the translation of FolateScan into the OA clinic for non-invasive monitoring of disease activity as well as therapeutic efficacy. Second, there are several tissues that cannot be categorized as either OA or RA at the early stage of arthritis. The unique staining pattern of FR-β in the lining layer in OA patients will help to distinguish OA from RA. Third, it is generally thought that M2 macrophages participate in tissue repair/regeneration (9, 10). Given that synovial FR-β+ macrophages expressed a mixed pattern of M1 and M2 markers, the expression profiles of FR-β-expressing macrophages in OA synovial tissues cause concern that drugs targeting FR-β+ macrophages might prevent their anti-inflammatory function.

In conclusion, we have demonstrated the expression of functional FR-β in synovial macrophages with a mixed expression pattern of M1 and M2 markers in patients with OA. Our data suggest that FR-β or CD163 is not an ideal marker for classifying M1 and M2 macrophages. Understanding the expression of FR-β in OA synovial tissues may lead to the development of promising tools for diagnosing and treating OA.

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