Folate Receptor β Is Expressed by Tumor-Associated Macrophages and Constitutes a Marker for M2 Anti-inflammatory/Regulatory Macrophages

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Abstract

Macrophage activation comprises a continuum of functional states critically determined by cytokine microenvironment. Activated macrophages have been functionally grouped according to their response to pro-Th1/proinflammatory stimuli [lipopolysaccharide, IFNγ, granulocyte macrophage colony-stimulating factor (GM-CSF); M1] or pro-Th2/anti-inflammatory stimuli [interleukin (IL)-4, IL-10, M-CSF; M2]. We report that folate receptor β (FRβ), encoded by the FOLR2 gene, is a marker for macrophages generated in the presence of M-CSF (M2), but not GM-CSF (M1), and whose expression correlates with increased folate uptake ability. The acquisition of folate uptake ability by macrophages is promoted by M-CSF, maintained by IL-4, prevented by GM-CSF, and reduced by IFNγ, indicating a link between FRβ expression and M2 polarization. In agreement with in vitro data, FRβ expression is detected in tumor-associated macrophages (TAM), which exhibit an M2-like functional profile and exert potent immunosuppressive functions within the tumor environment. FRβ is expressed, and mediates folate uptake, by CD163+CD68+CD14+ M2–IL-10–producing TAM, and its expression is induced by tumor-derived ascitic fluid and conditioned medium from fibroblasts and tumor cell lines in an M-CSF–dependent manner. These results establish FRβ as a marker for M2 regulatory macrophage polarization and indicate that folate conjugates of therapeutic drugs are a potential immunotherapy tool to target TAM. [Cancer Res 2009;69(24):9395–403]

Introduction

Macrophages exhibit a continuum of functional activation states under homeostatic and pathologic conditions (1, 2). Depending on the stimulus, activated macrophages acquire microbicidal, pro-inflammatory, and antitumor activities, but might also contribute to tissue repair, resolution of inflammation, and tumor cell growth and metastasis (1). These two extremes of the spectrum of macrophage activation have been coined as “classic”/M1 and “alternative”/M2 (3) and play opposing roles during immune and inflammatory responses. Although granulocyte macrophage colony-stimulating factor (GM-CSF) and M-CSF contribute to macrophage differentiation, each cytokine promotes the acquisition of distinct pathogen susceptibility (4) and inflammatory functions (5–8). GM-CSF–derived macrophages (M1) are proinflammatory and potentiate Th1 responses, whereas M-CSF–driven macrophages (M2) secrete IL-10 in response to pathogens and do not activate Th1 responses (8).

Tumor-associated macrophages (TAM) are abundant immunosuppressive cells recruited into the tumor microenvironment by cytokines such as M-CSF and CCL2 (9). The relevance of M-CSF and TAM in tumor progression and metastasis is now well established (10, 11). TAM represent a unique type of M2-polarized macrophages, as they promote angiogenesis, tissue remodeling, and repair (2, 12). In fact, clinical studies have revealed a correlation between high tumor macrophage content and poor patient prognosis. Because TAM are potential targets for anticancer therapy (13, 14), identification of TAM-specific markers constitutes a very active area of research.

The folate receptor gene family includes four members (FRα or FOLR1, FRβ or FOLR2, FRγ or FOLR3, and FRδ or FOLR4), whose encoded products bind folic acid with high affinity (15). FOLR1 and FOLR2 encode glycosyl phosphatidylinositol–anchored endocytic receptors expressed in certain epithelial tissues and various tumors (FOLR1; refs. 16, 17) or in normal myeloid cells and acute myelogenous leukemias (FOLR2; refs. 18–20). Within the myeloid lineage, folate receptor β (FRβ) is expressed in a nonfunctional state in CD34+ bone marrow cells (21, 22) and neutrophils (18), whereas it mediates folate binding in activated synovial macrophages from rheumatoid arthritis (23) and in ovarian cancer–associated murine macrophages (24). The high affinity of FRα and FRβ for folate binding, their endocytic capacity, and their restricted expression have prompted the evaluation of the potential therapeutic value of folate-drug conjugates in cancer and inflammatory pathologies (25, 26).

In the present article, we describe that functional FRβ is specifically expressed by M-CSF–polarized (M2) macrophages as well as by ex vivo isolated TAM, and that tumors induce its expression in an M-CSF–dependent manner, thus supporting folate-drug conjugates as valuable tools to target TAM in tumor immunotherapy protocols.

Materials and Methods

Cell culture and treatments. Human monocytes were purified by magnetic cell sorting using CD14 microbeads (Miltenyi Biotech) as described (27).
M1 or M2 macrocyte-derived macrophages were generated in the presence of GM-CSF (1,000 units/mL, ImmunoTools GmbH) or M-CSF (10 ng/mL), respectively. When indicated, macrophages were treated for 72 h with IL-6 (1,000 units/mL, ImmunoTools GmbH) or M-CSF (10 ng/mL), or lipopolysaccharide (LPS; 50 ng/mL; *E. coli* 055:B5, Sigma) for 48 h. Human tumor cell lines (JAR, JEG-3, NIH-OVCAR-3, and Colo320) were cultured in DMEM containing 10% FCS. Cultures of tumor-associated fibroblasts were done as reported (26). Flow cytometry on permeabilized TAM was done using phycoerythrin (PE)-labeled anti-CD68 monoclonal antibody (clone Y1/82A, Biolegend), Alexa Fluor 647 labeled anti-Tie2 monoclonal antibody (clone RM3/1, Biolegend), and a polyclonal antiserum rabbit affinity-purified antibody. The presence of Tie2-positive FRβ was determined by indirect immunofluorescence (28) using rabbit polyclonal antisera against human FRβ (ref. 18; empty histogram), as a control (filled histogram), a previously described rabbit preimmune antiserum (29) was used. C. FRβ expression in M1 and M2 macrophages, as shown by binding (4°C) and uptake (37°C) of folate-FITC (empty histogram, black line). Transferrin-FITC internalization (empty histogram, gray line) was determined in parallel on both macrophage types. Each experiment was done three times, and a representative experiment is shown. D. binding (4°C) and internalization (37°C) of folate-FITC by M2 macrophages, in the absence (empty histograms, black line) or the presence (empty histograms, gray line) of a 100 mol/L excess of folic acid. The experiment was done four times, and one of the experiments is shown. Representative confocal sections of M2 macrophages incubated with folate FITC for 1 h at 37°C, and their corresponding differential interference contrast images, are shown. The percentage of marker-positive cells and the mean fluorescence intensity (in parentheses) are indicated in flow cytometry experiments (B–D), and filled histograms indicate cell autofluorescence (C and D).

Western blot. Western blot was carried out with 10 μg of lysates from crude plasma membranes (30). Protein detection was done with a polyclonal antisera against FRβ (18) or a monoclonal antibody against CD29. For control purposes, a previously described rabbit pre-immune antiserum was used (29).

PCR. Total RNA from solid tumor tissue and TAM was extracted (RNaseasy kit, Qiagen), retrotranscribed, and amplified using standard procedures. Oligonucleotides specific for FOLR2, MAFB, IL10, ESR1, MAGEA3, and GAPDH were as follows: FRBs, 5′-AGAAGACCATGGTCTGGAAATGGATG-3′, and FRBs, 5′-GACTGACTACGCAAGGACCCAGTT-3′ (21); Maf-Bs, 5′-CCGGCTGGCCGGAGAC-3′, and Maf-Bs, 5′-CTAGGAGGCCGCTGCGT-3′ (31); IL10s, 5′-ATGCCCAAAGCTGAGAACCAA-3′, and IL10bs, 5′-TCAGTTACGAGCGCCAG-3′; ESR1s, 5′-TCAGTTACGAGCGCCAG-3′, and ESR1bs, 5′-GGCTACGATCCACCAAGG-3′; MAGEA3s, 5′-GAAGGCGGGCCAGGACAG-3′, and MAGEA3bs, 5′-GGAGTACTCCATAGGATTTGGCTCC-3′; and GAPDHs, 5′-GGTGGAGACGGGAAGGTTGCT-3′, and GAPDHbs, 5′-CGCCTACACGCCACATTTT-3′. Amplified fragments (783 bp for FOLR2, 347 bp for MAFB, 352 bp for IL10, 511 bp for ESR1, 457 bp for GAPDH, and 423 bp for MAGEA3) were resolved by agarose gel electrophoresis. For quantitative reverse transcription-PCR (RT-PCR), oligonucleotides for FOLR1, FOLR2, FOLR3, JDP2, NRAMP1, and IL10 were designed according to the Roche software for quantitative real-time PCR, and RNA was amplified using the Universal Human Probe Roche library (Roche Diagnostics). Assays...
were made in triplicates and results normalized according to the expression levels of 18S RNA and GAPDH. Results were obtained using the ΔΔCt method for quantitation and expressed as normalized fold expression.

Confocal microscopy and immunohistochemistry. Human melanoma tissues (subcutaneous tissue, lymph node, and lung metastasis) were obtained from patients with primary and metastatic lesions undergoing surgical treatment. Thick sections (4 μm in depth) of cryopreserved tissue were first blocked for 10 min with 1% human immunoglobulins and then incubated for 1 h with a rabbit polyclonal antiserum against human FRβ (18), anti-CD163 or HMB-45 monoclonal antibodies, or isotype-matched control antibodies. All primary antibodies were used at 1 to 5 μg/mL, followed by incubation with FITC-labeled antimouse and Texas red-labeled antirabbit secondary antibodies. Samples were imaged using a confocal scanning inverted A OBS/SP2 microscope (Leica Microsystems) with a 63× PL-APo NA 1.3 immersion objective. Image processing and colocalization analyses (scatter plots) were assessed with the Leica Confocal Software LCS-15.37. Tissue microarrays (TM Ah-MTC-01, RayBiotech) were processed according to the manufacturer’s recommendations.

Results

FRβ is expressed in macrophages generated in the presence of M-CSF. Gene expression profiling on macrophages generated in the presence of GM-CSF (M1) or M-CSF (M2) resulted in the identification of more than 250 differentially expressed genes (>2-fold differences, P < 0.05; data not shown). Among them, FOLR2, which codes for FRβ, was preferentially expressed in M2 macrophages (P = 1.3 × 10⁻³; Fig. 1A). The JDP2 gene, which encodes an activator protein-1 repressor, also showed higher expression in M2 macrophages (P = 0.002), whereas SLC11A1, which encodes the NRAMP1 protein associated with classic macrophage activation, was expressed at higher levels in M1 macrophages (P = 0.029; Fig. 1A). Interestingly, and in agreement with their anti-inflammatory activity, the expression of IL10 was considerably higher in M2–primed macrophages (P = 1.2 × 10⁻⁴). The differential expression of FOLR2, JDP2, SLC11A1, and IL10 in both types of macrophages was confirmed by real-time RT-PCR on mRNA from independent donors (Fig. 1A). Besides, FRβ expression was exclusively detected in membrane lysates and on the cell surface of M2 macrophages (Fig. 1B), thus validating the transcriptome data.

Because FRβ binds folic acid and folate conjugates (32), the ability of FRβ to mediate folate-FITC uptake by M2 macrophages was assessed. Whereas both macrophage types endocytosed transferrin-FITC, M-CSF–polarized macrophages displayed folate binding and internalization ability, and GM-CSF–induced macrophages showed no folate uptake capacity, in agreement with their lack of FRβ expression (Fig. 1C). Folate binding and uptake by M-CSF macrophages were specific, as both were inhibited by a 100 μM/L excess of folic acid (Fig. 1D). Moreover, folate conjugates entered cells by endocytosis because most of the folate-FITC fluorescence could not be stripped from the cell surface by an acid wash step (Supplementary Fig. S1). Considering that neither FOLR1 nor FOLR3 was expressed by M-CSF macrophages (Supplementary Fig. S2), FOLR2-encoded FRβ protein must be responsible for the folate binding ability of M2 macrophages. Kinetic studies revealed that FOLR2 mRNA and FRβ protein are initially detected 48 to 72 hours after M-CSF addition, and that their levels dramatically increase at later incubation times (Fig. 2A and B). Acquisition of folate uptake ability correlated with protein expression at all time points and showed its highest level at the end of the culture period (Fig. 2C). Therefore, M-CSF promotes the expression of a functional FRβ protein, which constitutes a marker of M-CSF–polarized M2 macrophages.

Expression of FRβ in TAM. TAM are an M2-skewed macrophage population that exhibits immunosuppressive activity within the tumor microenvironment, and whose recruitment and differentiation is influenced by M-CSF (9). Given the preferential expression of FRβ in M-CSF–polarized M2 macrophages, its presence was evaluated in TAM. Immunohistochemistry revealed that FRβ is frequently coexpressed with CD163 in TAM from primary and metastatic melanoma (Figs. 3A and 4A) but is absent from melanoma HMB-45⁺ cells (Figs. 3A and 4A). In fact, FOLR2 mRNA could be detected in three melanoma samples (Fig. 3B). Ex vivo isolated CD14⁺ TAM from the pleural fluid of a metastatic melanoma...
expressed mRNA for FOLR2, IL10, and the macrophage-specific MAFB (31), whereas they lacked expression of the melanoma-specific marker MAGEA3 mRNA (Fig. 3B, lanes 4) and were devoid of FOLR1 and FOLR3 mRNA (Supplementary Fig. S3). Three-color analysis on isolated melanoma TAM indicated that all FRβ+ macrophages are CD68+ and that the percentage of FRβ+ CD163+ macrophages (87%) is similar to that of CD163+ CD68+ cells (88%; Fig. 3C). Thus, most melanoma TAM from the analyzed sample coexpress CD163, CD68, and FRβ and exhibit folate-FITC internalization ability (Fig. 3D). It is also worth noting that a percentage of FRβ+ macrophages coexpress Tie2 (36%; Fig. 3C). Altogether, these results indicate that FRβ is functionally expressed on IL10 mRNA–expressing CD14+ CD68+ CD163+ melanoma TAM.

Evaluation of FRβ expression on other tumor tissues indicated that FRβ is detected in the stroma of lung, ovary, colon, gastric, and breast cancers, where numerous CD68+ TAM were also present (Fig. 4B). Analysis of ex vivo isolated CD14+ TAM from a metastatic breast adenocarcinoma also revealed the coexpression of CD68 and FRβ, and that 80% of the cells exhibited a CD163+ FRβ+ phenotype (Fig. 5A). Importantly, primary and metastatic breast adenocarcinoma tissues were found to contain both FOLR2 and MAFB mRNA (Fig. 5B), and ex vivo isolated CD14+ metastatic breast adenocarcinoma TAM expressed FOLR2, IL10, and MAFB mRNA (Fig. 5B, lane 5). CD14+ CD163+ TAM also exhibited specific folate-FITC binding and uptake (Fig. 5C) and produced IL-10 in response to LPS stimulation (Fig. 5D). Because FOLR2 and IL10 mRNA are coexpressed in M2 macrophages in vitro (Fig. 1), and TAM from metastatic breast adenocarcinoma express functional FRβ and produce IL-10, these results indicate that FRβ activity marks anti-inflammatory M2-like TAM.

**Parameters affecting FRβ expression on human macrophages.** GM-CSF and M-CSF are tumor-derived factors that modulate myeloid cell differentiation (33). Unlike M-CSF, GM-CSF abrogated the acquisition of FOLR2 mRNA during in vitro monocyte-to-macrophage differentiation, even in the presence of M-CSF (Fig. 6A). This result explains the differential expression of FRβ on both types of macrophages, and suggests that the relative levels of tissue GM-CSF and M-CSF determine macrophage FRβ expression. Other cytokines commonly released by tumors (33) also affected FOLR2 mRNA: IL-6 alone and IL-10 in combination with M-CSF upregulated FOLR2 mRNA expression (Fig. 6B). Therefore, tumor-derived cytokines (M-CSF, GM-CSF, IL-6, and IL-10) modulate FRβ expression in human macrophages.
Expression of FRβ on TAM led us to analyze the nature of the stimuli that might control its presence in the tumor microenvironment. FOLR2 mRNA was variably upregulated by supernatants from tumor cell lines, with placenta choriocarcinoma JAR and JEG-3 cells and ovary carcinoma NIH-OVCAR-3 cells promoting the highest level of upregulation (Fig. 6C). In contrast, conditioned media from colon carcinoma Colo320 cells had no effect (Fig. 6C). More importantly, ascitic fluid from the breast carcinoma analyzed in Fig. 5 promoted a strong upregulation of FOLR2 mRNA (Fig. 6C), confirming that tumor cells release factors that upregulate human macrophage FRβ expression. The addition of a blocking anti-M-CSF monoclonal antibody greatly reduced the upregulation of FOLR2 mRNA promoted by ascitic fluid from breast carcinoma (Fig. 6D) or by conditioned medium from tumor-associated fibroblasts or JEG-3 tumor cells (Fig. 6D). Therefore, M-CSF is a major determinant for FRβ expression on human macrophages and contributes, alone or in combination with other cytokines, to FRβ cell surface expression on TAM.

Discussion

GM-CSF and M-CSF contribute to the generation of different macrophage subsets and enhance myeloid cell survival and proliferation (9). However, GM-CSF promotes the generation of myeloid cells with potent antigen presentation activity, whereas M-CSF leads to the generation of macrophages with regulatory properties (9). Gene expression profiling allowed us to identify FRβ as preferentially expressed by macrophages generated under the influence of M-CSF, which display FRβ-dependent folate binding ability. FRβ expression on in vitro differentiating macrophages was enhanced by M-CSF and by tumor cell-conditioned medium in an M-CSF-dependent manner. Conversely, GM-CSF prevented the acquisition of FRβ expression. Importantly, FRβ was detected in TAM, where FRβ-mediated folate binding activity correlates with the presence of IL10 mRNA. Therefore, FRβ constitutes a marker for M-CSF–primed IL-10–expressing M2-polarized macrophages, providing a molecular basis for the value of folate-conjugated drugs in cancer therapy approaches.

Figure 4. Expression of FRβ in TAM from primary and metastatic melanoma. A, expression of FRβ in melanoma-infiltrating macrophages on a primary melanoma (#130, bottom) or two metastatic melanomas (#98 and #146, top and middle), as determined by double immunofluorescence analysis of FRβ and the macrophage marker CD163 (top rows) or FRβ and the melanoma tumor marker HMB-45 (bottom rows). B, expression of FRβ in tumors of distinct tissue origins. Light microscopy images of the macrophage marker CD68 (middle) and FRβ (right) staining of tumor tissue from lung squamous cancer (1; magnification, ×20), ovarian cystadenoma-mucous (2; magnification, ×20), rectal colon adenocarcinoma (3; magnification, ×20), gastric adenocarcinoma (4; magnification, ×40), and breast invasive ductal cancer (5; magnification, ×40). Left, staining yielded by normal rabbit serum, used as a control.
Because macrophage polarization is stimulus dependent (1), alternatively activated M2 macrophages have been further classified as M2a, M2b, or M2c in an effort to link genetic markers to specific macrophage-activating stimuli (34). The expression of FRβ in M-CSF–generated macrophages indicates that it is preferentially expressed by IL-10–producing M2 macrophages and, therefore, identifies a population of macrophages with anti-inflammatory/ regulatory properties. The presence of FRβ in M-CSF–primed in vitro macrophages is in agreement with its upregulation in human decidual macrophages, which exhibit an immunosuppressive phenotype and whose gene expression profile closely corresponds to that of M2-polarized macrophages (35). Further supporting its presence on M2 macrophages, FRβ has been detected on F4/80+ CD68+ murine peritoneal macrophages (36), where its mRNA levels can be further upregulated by IL-4 (37). Therefore, FRβ expression seems not to be restricted to anti-inflammatory/regulatory IL-10– producing M2 macrophages and marks a wider range of alternatively activated macrophages in the human and murine systems. However, the functional state of FRβ on murine peritoneal macrophages is still not clear because folate binding ability is only detected after stimulation with inflammatory stimuli (26).

The expression of FRβ on TAM from primary and metastatic melanoma and breast carcinoma (Figs. 3–5) is also in agreement with a previous report describing the presence of FRβ in CD68+ CD163+ cells within human and rat glioblastoma (36). In an apparent contradiction, gene expression profiling has revealed downregulated FRβ mRNA levels in murine fibrosarcoma TAM relative to the levels detected in thioglycollate-elicited peritoneal macrophages (12). However, because the latter exhibit functional characteristics of M-CSF–driven M2 macrophages (38), these results do not rule out the presence of detectable levels of FRβ in murine TAM. Besides, it is also possible that FRβ is expressed by TAM in a tumor-dependent manner, a phenomenon which would be in agreement with its differential upregulation by distinct tumor-conditioned media (Fig. 6) and the variable levels of FRβ in TAM from a variety of human tumors (Fig. 4). Finally, it is also possible that differences might exist between murine and human TAM, as it is already evident that paradigmatic M2 murine macrophage markers (Arginase and Ym1) are not useful to identify human alternatively activated macrophages (39). Whether the acquisition of FRβ expression by tumor-infiltrating macrophages is detrimental for the tumor (e.g., by removing folate) or favors tumor cell growth is a matter that deserves further investigation. Regardless of the precise role of FRβ on TAM, the presence of FRβ on their cell surface provides an opportunity for depletion of TAM through the use of folate-conjugated drugs. As an example, and while this article was being completed, Nagai and co-workers have shown the feasibility of reducing tumor growth by

![Figure 5](cancersres.aacrjournals.org)
targeting an immunotoxin to TAM using an antineuse FRβ monoclonal antibody (36).

Given the tumor influence on macrophage functions (40), FRβ might specifically mark tumor-infiltrating human macrophages whose effector functions have been already skewed by tumor-derived factors. In this regard, our data also suggest that tumor-derived M-CSF, which recruits and shapes macrophage functions (33), would be the primary determinant for FRβ expression. However, FRβ expression is also detected in resident macrophages within nontumor tissue (data not shown). This fact, together with the increase in FRβ expression during the in vitro macrophage differentiation that takes place in the absence of exogenous cytokines, might indicate that FRβ could be a macrophage differentiation marker under homeostatic conditions, and whose levels could be maintained or upregulated by anti-inflammatory cytokines and downregulated by pro-inflammatory stimuli. In this regard, cytokines such as IL-4, IL-13, and IL-10, which promote macrophage alternative activation, trigger a transient increase of FOLR2 mRNA levels in M2 macrophages (Supplementary Fig. S44 and B). By contrast, LPS greatly downregulates FOLR2 mRNA levels (Supplementary Fig. S44 and B) although it does not lead to a great decrease in cell surface FRβ (Supplementary Fig. S4C). This divergence might be explained by the fact that FOLR2 is an endocytic receptor whose protein levels are higher than those present on the cell surface. In fact, flow cytometry on permeabilized cells showed that a large proportion of FRβ is located intracellularly in both in vitro M2 macrophages and TAM (Supplementary Fig. S5).

The presence of functional FRβ on M-CSF–primed macrophages and the detection of FRβ mRNA in other types of M2-polarized macrophages (12, 35, 37) are difficult to reconcile with its expression (25) and function (26) in synovial macrophages from rheumatoid arthritis patients, which are embedded in an inflammatory pro-M1 environment. It could be speculated that synovial macrophages might exhibit a mixed M1/M2 phenotype, similar to what occurs with myeloid populations within tumors (41), an explanation that would be compatible with the high levels of M-CSF expression.

Figure 6. Parameters affecting FRβ expression on human macrophages. A and B, FOLR2 mRNA expression in macrophages exposed for 72 h to the indicated cytokines, as determined by quantitative RT-PCR. Results are expressed as normalized fold expression relative to 18S rRNA levels and the FOLR2 RNA levels in peripheral blood monocytes (Mon.). Columns, mean of triplicate determinations; bars, SD. C, FOLR2 mRNA expression in macrophages exposed for 72 h to conditioned media from the asctic fluid of a breast carcinoma (ABC) or tumor cell lines, as determined by quantitative RT-PCR. Results are expressed as normalized fold expression (relative to 18S rRNA levels). Columns, mean of triplicate determinations from three independent macrophage preparations; bars, SD. D, inhibitory effect of anti–M-CSF on FOLR2 mRNA levels induced by asctic fluid from metastatic breast carcinoma (ABC) or conditioned-medium from JEG-3 placenta chorionic carcinoma (JEG-3) or tumor-associated fibroblasts (Fibroblast). The results are depicted as the FOLR2 mRNA levels detected in the presence of the anti–M-CSF antibody relative to the levels seen in untreated cells (set to 100 in the three cases).
present in rheumatoid arthritis synovia (42). M-CSF is produced by synovial fibroblasts, and administration of M-CSF is known to exacerbate arthritis in some settings (9, 43). Therefore, the levels of M-CSF within the synovia of rheumatoid arthritis might suffice to promote FRβ expression on surrounding macrophages, although the concomitant presence of extremely high levels of tumor necrosis factor α might override its immunosuppressive actions. Alternatively, because M-CSF contributes to macrophage recruitment, FRβ expression might mark macrophages newly recruited into the arthritic synovia, whose later levels of FRβ expression would be determined by the pro-inflammatory environment. In this regard, it is worth noting that (a) FRβ+ macrophages are more prominently detected at early stages during development of animal models of atherosclerosis and muscle injury and in rheumatoid arthritis in humans (b) in vitro acute (48 hours) exposure of FRβ-expressing M2 macrophages to M1-polarizing stimuli (e.g., LPS, GM-CSF, and IFNγ) does not result in loss of FRβ expression, which is only moderately downregulated by IFNγ (Supplementary Fig. S4C). Therefore, folate-targeted killing of FRβ+ macrophages in inflammatory disease murine models might contribute to inflammation resolution by preferentially eliminating newly recruited macrophages.

Although further studies are needed to correlate FRβ expression and function in macrophages within inflamed tissues, our results indicate that cytokines favoring the generation of anti-inflammatory/ regulatory macrophages, and known to shape TAM effector functions (M-CSF and IL-10), promote and are permissive for FRβ expression, whereas factors skewing macrophage polarization toward the proinflammatory branch either inhibit (IFNγ) or abrogate FRβ expression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References


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