Arthritis & Rheumatology
Vol. 73, No. 11, November 2021, pp 2003–2014
DOI 10.1002/art.41792

American College of Rheumatology
Empowering Rheumatology Professionals

Interleukin-34 Reprograms Glycolytic and Osteoclastic Rheumatoid Arthritis Macrophages via Syndecan 1 and Macrophage Colony-Stimulating Factor Receptor

Katrien Van Raemdonck,[1] Sadiq Umar,[1] Karol Palasiewicz,[1] Michael V. Volin,[2] Hatem A. Elshabrawy,[3] Bianca Romay,[4] Chandana Tetali,[4] Azam Ahmed,[4] M. Asif Amin,[5] Ryan K. Zomorrodi,[4] Nadera Sweiss,[4] and Shiva Shahrara[1]

Objective. In rheumatoid arthritis (RA), elevated serum interleukin-34 (IL-34) levels are linked with increased disease severity. IL-34 binds to 2 receptors, macrophage colony-stimulating factor receptor (M-CSFR) and syndecan 1, which are coexpressed in RA macrophages. Expression of both IL-34 and syndecan 1 is strikingly elevated in the RA synovium, yet their mechanisms of action remain undefined. This study was undertaken to investigate the mechanism of action of IL-34 in RA.

Methods. To characterize the significance of IL-34 in immunometabolism, its mechanism of action was elucidated in joint macrophages, fibroblasts, and T effector cells using RA and preclinical models.

Results. Intriguingly, syndecan 1 activated IL-34–induced M-CSFR phosphorylation and reprogrammed RA naive cells into distinctive CD14+CD86+GLUT1+ M34 macrophages that expressed elevated levels of IL-1β, CXCL8, and CCL2. In murine M34 macrophages, the inflammatory phenotype was accompanied by potentiated glycolytic activity, exhibited by transcriptional up-regulation of GLUT1, c-Myc, and hypoxia-inducible factor 1α (HIF-1α) and amplified pyruvate and L-lactate secretion. Local expression of IL-34 provoked arthritis by expanding the glycolytic F4/80-positive, inducible nitric oxide synthase (iNOS)–positive macrophage population, which in turn attracted fibroblasts and polarized Th1/Th17 cells. The cross-talk between murine M34 macrophages and Th1/Th17 cells broadened the inflammatory and metabolic phenotypes, resulting in the expansion of IL-34 pathogenicity. Consequently, IL-34–instigated joint inflammation was alleviated in RAG$^{-/-}$ mice compared to wild-type mice. Syndecan 1 deficiency attenuated IL-34–induced arthritis by interfering with joint glycolytic M34 macrophage and osteoclast remodeling. Similarly, inhibition of glycolysis by 2-deoxy-D-glucose reversed the joint swelling and metabolic rewiring triggered by IL-34 via HIF-1α and c-Myc induction.

Conclusion. IL-34 is a novel endogenous factor that remodels hypermetabolic M34 macrophages and facilitates their cross-regulation with T effector cells to advance inflammatory bone destruction in RA.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disorder characterized by an abundance of inflammatory cytokines, propagating immune cell infiltration, painful joint swelling, and late-stage cartilage and bone erosion (1). Macrophages contribute substantially to this cytokine-rich inflammatory milieu (2). Independent of the underlying cause of RA, activated macrophages are highly involved in pannus remodeling and radiologic progression (3,4). RA standard of care therapies primarily target tumor necrosis factor (TNF) and interleukin-6 (IL-6) function; however, up to 50% of patients either do not respond or lose their responsiveness to these therapies over time.

Supported in part by the Department of Veteran's Affairs (MERIT award BX002286), the NIH (grants AI-147697, AR-056099, and AR-065778), the National Psoriasis Foundation, the Pfizer Investigator-Initiated Research Program, and the Chicago Biomedical Consortium Accelerator award.

[1]Katrien Van Raemdonck, PhD, Sadiq Umar, PhD, Karol Palasiewicz, BS, Shiva Shahrara, PhD: Jesse Brown VA Medical Center and The University of Illinois at Chicago; [2]Michael V. Volin, PhD: Midwestern University, Downers Grove, Illinois; [3]Hatem A. Elshabrawy, PhD: Sam Houston State University, Conroe, Texas; [4]Bianca Romay, BS, Chandana Tetali, BS, Azam Ahmed, Ryan K. Zomorrodi, Nadera Sweiss, MD: The University of Illinois at Chicago; [5]M. Asif Amin, MD: University of Michigan Medical School, Ann Arbor.

No potential conflicts of interest relevant to this article were reported.

Address correspondence to Shiva Shahrara, PhD, The University of Illinois at Chicago, Department of Medicine, Division of Rheumatology, 840 S Wood Street, CSB Suite 1114, Chicago, IL 60612. Email: shahrara@uic.edu.

Submitted for publication September 21, 2020; accepted in revised form April 27, 2021.

Earlier studies have shown that RA naive CD4+ T cells undergo a distinct metabolic reconfiguration, shifting from glucose metabolism to the pentose phosphate pathway, which supports a biased Th1/Th17 polarization (5,6). RA fibroblasts activated with platelet-derived growth factor (PDGF) or TNF can increase glucose metabolism via glycolysis and oxidative phosphorylation (7–9). Studies in cancer, atherosclerosis, and obesity have revealed that polarization of classically activated (M1) macrophages promotes metabolic reprogramming toward glycolysis and away from oxidative phosphorylation (10,11). Recent reports elucidated that hypermetabolic $ATP^{high}ROS^{high}$ macrophages are characterized by hyperglycolysis, which is linked to the expansion of glucose transporters and glycolytic enzymes (12,13). Yet, the endogenous factors and the mechanism by which RA macrophages are reprogrammed remain unclear.

Recently, IL-34 has gained interest as a novel biomarker for RA (14). Serum levels of IL-34 correlate with several RA markers, including C-reactive protein level, erythrocyte sedimentation rate, rheumatoid factor, and anti–cyclic citrullinated peptide antibody level (15). A correlation was also found between synovial IL-34 expression and the accumulation of leukocytes in RA synovial fluid (SF) (14). Additionally, IL-34 expression and function have been implicated in other diseases, such as breast and lung cancer, liver fibrosis, type 2 diabetes mellitus, and cardiovascular dysfunction (16,17). The mechanism of action of IL-34 has predominantly been attributed to macrophage colony-stimulating factor receptor (M-CSFR), a receptor shared with M-CSF. M-CSF binding to M-CSFR cultivates immunosuppressive alternatively activated (M2) macrophages (18,19). In contrast, the impact of IL-34 on the macrophage profile remains unresolved. Conflicting reports support either an inflammatory (20) or an immunosuppressive (21,22) phenotype for IL-34–differentiated (M34) macrophages. This controversy carries over to the indirect effect of IL-34 on T effector cell polarization. IL-34 has been reported to stimulate Th17 cell polarization, via IL-1α and IL-6, thereby aggravating autoimmune RA pathogenesis (15,20,23). In contrast, others have demonstrated that the immunoregulatory macrophages polarized by IL-34 promote transplant tolerance by expanding the FoxP3+ Treg cell population (24).

Interestingly, syndecan 1, a heparan sulfate proteoglycan that functions as a co-receptor of M-CSFR, modulates the biologic activity of IL-34 (25). Since M-CSF does not bind to syndecan 1, its involvement could account for a distinct pathway that is unique to IL-34–mediated pathology. Although an extensive number of studies have shown that syndecan 1 potentiates tumorigenesis by regulating cancer cell invasion, survival, and metastasis (26,27), its pathogenic role in RA is undetermined.

We investigated the arthritogenic potential of IL-34 and syndecan 1 and their impact on RA immunometabolism. It was observed that in RA macrophages, syndecan 1 triggers M-CSFR phosphorylation upon IL-34 binding. IL-34 rewired naive cells into glycolytic CD14+CD86+GLUT1+ M34 macrophages, which were disrupted by M-CSFR or syndecan 1 blockade. In $SDC\text{-}1^{-/-}$ and $RAG^{-/-}$ mice, dysregulation of the reprogramming of M34 macrophages and their deficient cross-talk with Th1/Th17 cells reversed IL-34–elicited joint inflammation and osteoclast formation. Furthermore, 2-deoxy-D-glucose (2-DG) therapy confirmed that M34 macrophage metabolic remodeling via hypoxia-inducible factor 1α (HIF-1α) and c-Myc may be indispensable for IL-34–induced arthritis. In short, restricting the function of IL-34 or its co-receptors may provide a promising therapeutic strategy for RA patients.

MATERIALS AND METHODS

Patient samples. Peripheral blood and SF samples were collected according to our protocol approved by The University of Illinois at Chicago Institutional Ethics Review Board. Normal subjects, patients with osteoarthritis (OA), and patients with RA participated in this study after providing informed consent. RA patients were diagnosed according to the American College of Rheumatology 1987 revised criteria (28). Human peripheral blood mononuclear cells were isolated by density-gradient centrifugation using Ficoll-Paque Premium. Monocytes were negatively selected using an EasySep human monocyte enrichment kit (StemCell Technologies) according to the manufacturer's instructions. Monocytes were cultured in 20% fetal bovine serum (FBS)/RPMI for 2–3 days to obtain in vitro differentiated naive macrophages. RA fibroblast-like synoviocytes (FLS) were isolated from fresh RA synovial tissue (ST) digested with Dispase/Collagenase/DNase, as previously described (29,30). More detail about the study design, methods, and the antibodies used for flow cytometry, immunohistochemistry, and Western blotting are provided in the Supplementary Methods, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41792/abstract.

Western blot analysis. Cell lysates from in vitro differentiated macrophages, peripheral blood T cells, or RA FLS were probed for syndecan 1 and M-CSFR to validate receptor expression. Actin was detected to confirm equal loading. Blots were probed for phosphorylated signaling molecules or activated phosphorylated M-CSFR (Y723) compared to either actin or GAPDH.

Flow cytometric analysis. Negatively selected monocytes were differentiated in vitro and stimulated with IL-34 (300 ng/ml) or left untreated (control) for 24 hours before staining with fluorescein isothiocyanate–conjugated CD14, allophycocyanin-conjugated CD86, and phycoerythrin (PE)–Cy7–conjugated CD206 antibodies or DAPI. Viable (DAPI-negative) cells were analyzed for double-positive CD14+CD86+, CD14+CD206+, and CD14+GLUT1+ populations. Untreated, viable (DAPI-negative) RA FLS were stained with PE-conjugated protein tyrosine phosphatase receptor type Z1 (PTPRZ1) antibody to determine baseline PTPRZ1 surface expression. Antibody concentrations

are indicated in Supplementary Table 1, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41792/abstract. Supplementary Figure 1, available on the *Arthritis & Rheumatology* website at http://onlinelibrary. wiley.com/doi/10.1002/art.41792/abstract, shows the gating strategy used.

Seahorse cell energy phenotype test. Glycolytic capacity (extracellular acidification rate [ECAR]) and oxygen consumption (oxygen consumption rate [OCR]) were measured using a Seahorse XF Cell Energy Phenotype Test kit (Agilent Technologies), according to the manufacturer's instructions, in RAW 264.7 cells (5×10^3 cells/well) treated with control (phosphate buffered saline [PBS]) or IL-34. Cells were preconditioned with the stimuli in 0% FBS/Dulbecco's modified Eagle's medium for 24 hours before ECAR and OCR assessment.

Osteoclastogenesis assay. Osteoclastogenesis was evaluated following the previously described protocol (31). Bone marrow (BM)–derived myeloid precursors (4 days of culture with M-CSF) cultured in 10% FBS/α-minimum essential medium were either left untreated (control) or conditioned with suboptimal levels of RANKL/M-CSF (both 15 ng/ml), with or without IL-34 (300 ng/ml). Stimulation media was refreshed 2 times per week for 2 weeks and tartrate-resistant acid phosphatase (TRAP)–positive osteoclasts were stained (387A-1KT; Sigma-Aldrich).

Animals. All animal studies were approved by The University of Illinois at Chicago Animal Care and Use Committee. Wild-type (WT) and RAG1$^{-/-}$ (RAG$^{-/-}$) C57BL/6 mice were purchased from The Jackson Laboratory. SDC-1$^{-/-}$ mice were generated as previously described (26) and kindly provided by Dr. Caroline Alexander (University of Wisconsin–Madison). Mice (ages 8 weeks or older) were given intraarticular (IA) injections of control adenovirus (Ad-Control) or Ad-IL-34 (3×10^{10} viral particles/ankle), on days 0, 7, and 14. Mice treated with 2-DG were injected intraperitoneally with 7.5 mg/kg body weight 2-DG on days 0, 3, 7, 9, 11, 14, and 15. Mice were euthanized on different days as specified in the figure legends.

Immunohistochemistry. Formalin-fixed, paraffin-embedded human tissue samples were sectioned. Normal, OA, and RA ST samples were stained to quantify IL-34, M-CSFR, syndecan 1, and PTPRZ1 presentation. Staining was scored on a scale of 0–5 in a blinded manner, and distinguished within the synovial lining, sublining, and vasculature (29,32). Formalin-fixed mouse ankles were decalcified and paraffin-embedded. Slides were deparaffinized in xylene, and antigen retrieval was achieved as previously described (33). Mouse ankle sections were stained for macrophage markers (F4/80, inducible nitric oxide synthase [iNOS], and arginase 1), T cell marker (CD3), and fibroblast marker (vimentin), or for the transcription factors c-Myc and HIF-1α. Supplementary Table 1 specifies the antibodies used and their dilutions. The stained joint tissues were scored on a scale of 0–5 for inflammation, synovial lining thickness, and bone erosion (29,32).

Statistical analysis. GraphPad Prism software version 8 was used to generate figures and to perform statistical analysis. Bar graphs show the mean ± SEM. Box plots were used to visualize data distribution, from minimum to maximum with a center line showing the median. Where appropriate, individual data points are shown. Analysis of variance was first used to verify statistical significance when comparing multiple groups. Differences between the 2 groups were evaluated by an unpaired 2-tailed *t*-test unless otherwise specified.

RESULTS

Elevated expression of IL-34 and syndecan 1 in RA synovium. Experiments were conducted to characterize the expression and functional significance of IL-34 and its receptors in RA. Levels of IL-34, M-CSF, and granulocyte–macrophage colony-stimulating factor (GM-CSF) were quantified in plasma from normal controls, OA patients, and RA patients, as well as in OA SF and RA SF. Although IL-34 levels were comparable among all plasma samples, the IL-34 concentration was significantly higher (12 fold) in RA SF compared to OA SF, whereas M-CSF and GM-CSF levels did not differ significantly between RA SF and OA SF (Figure 1A). Histologic analysis substantiated the elevated expression of IL-34 in RA compared to OA and normal ST lining and sublining (Figure 1B and Supplementary Figure 2A, *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41792/abstract). However, M-CSFR expression was similar in the lining and sublining of all ST samples, with a modest increase observed in RA and OA vasculature (Figure 1C and Supplementary Figure 2B).

While M-CSFR is the primary receptor for IL-34, a co-receptor that modulates IL-34/M-CSFR signaling has been identified. We confirmed that IL-34 binds to the proteoglycan syndecan 1 (2.5 μg) with a 50% maximum response concentration of 3 ng/ml (Figure 1D). Histologic analysis revealed that syndecan 1 presentation was expanded in RA compared to OA and normal ST lining and sublining (Figure 1E and Supplementary Figure 2C). The endothelial distribution of syndecan 1 was similarly amplified in RA and OA ST compared to normal ST. Next, the expression of receptors for IL-34 was evaluated in RA macrophages, T cells, and FLS (Figure 1F). Unlike syndecan 1, which was expressed on RA macrophages, T cells, and RA FLS, M-CSFR expression was restricted to RA macrophages, as its extra- and intracellular domains were present only on these cells (140 and 52 kd). Fluorescence microscopy confirmed that IL-34 and its receptors, M-CSFR and syndecan 1, colocalize in RA ST, particularly in the lining layer (Supplementary Figure 2D). These results indicate that IL-34 levels are highly potentiated in RA SF and ST,

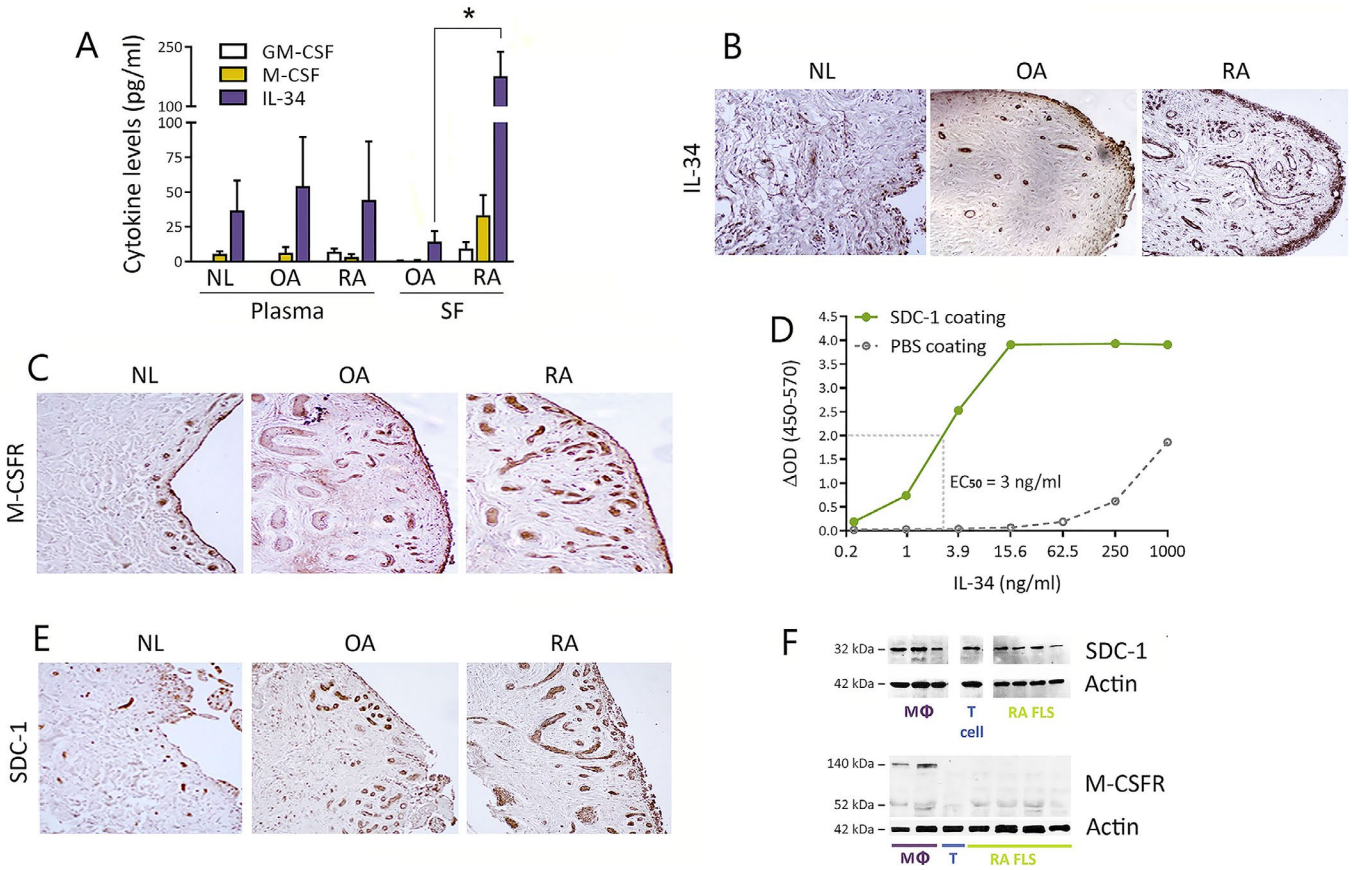

Figure 1. Expression of interleukin-34 (IL-34) and its receptors, macrophage colony-stimulating factor receptor (M-CSFR) and syndecan 1 (SDC-1), in specimens from normal (NL) controls, patients with osteoarthritis (OA), and patients with rheumatiod arthritis (RA). **A**, Granulocyte–macrophage colony-stimulating factor (GM-CSF), M-CSF, and IL-34 protein concentrations were determined in normal, OA, and RA plasma and synovial fluid (SF) samples. Bars show the mean ± SEM (n = 39 normal plasma samples, 10 OA plasma and 32 OA SF samples, and 39 RA plasma and 45 RA SF samples). * = $P < 0.05$. **B**, **C**, and **E**, Normal, OA, and RA synovial tissue samples were stained for IL-34 (n = 9 normal, 11 OA, and 9 RA samples) (**B**), M-CSFR (n = 9 normal, 11 OA, and 8 RA samples) (**C**), and syndecan 1 (n = 8 normal, 10 OA, and 10 RA samples) (**E**) and scored on a scale of 0–5 (Supplementary Figures 2A–C, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41792/abstract). Representative results are shown. Original magnification × 200. **D**, A biotinylated anti–IL-34 antibody was used to compare the amount of IL-34 retained on a recombinant syndecan 1–coated plate versus a phosphate buffered saline (PBS)–coated plate, both incubated with various doses of IL-34 (0.24–1,000 ng/ml). EC_{50} = 50% maximum response concentration. **F**, Western blot analysis was used to evaluate the expression of syndecan 1 (32 kd) and M-CSFR (full-length: 140 kd; intracellular domain: 52 kd) in RA peripheral blood macrophages (Mϕ), T cells, and fibroblast-like synoviocytes (FLS). Equal loading was confirmed by quantifying actin (42 kd).

and that macrophages, by expressing the syndecan 1/M-CSFR receptor complex, are the main effector cells that control IL-34 activity.

Immune cell extravasation and joint inflammation triggered by local IL-34 expression. Next, experiments were performed to evaluate the arthritogenic potential of IL-34. IA administration of IL-34 triggered progressive joint inflammation in mice (Figure 2A), along with exacerbated lining thickness and immune cell infiltration (Figure 2B and Supplementary Figure 3A, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41792/abstract). Histologic analysis revealed that IL-34–induced joint inflammation was accompanied by the accumulation of F4/80-positive macrophages, predominantly characterized as inflammatory iNOS-positive cells (Figure 2C and Supplementary Figure 3B). In contrast, the number of macrophages positive for F4/80 and arginase 1 was unchanged in mice with IL-34–induced arthritis relative to control mice. Consistent with these findings, mice with IL-34–induced arthritis had higher expression of monokines such as CCL5 (302 fold), IL-1β (36 fold), TNF (27 fold), CCL2 (24 fold), CXCL2 (20 fold), and IL-12 (4 fold) compared to control mice (Figures 2D and E).

In mice with IL-34–induced arthritis, the transcriptome data were validated by the increased joint protein levels of CCL5, IL-1β, CCL2, and IL-6 (12-fold to 1,407-fold higher than in control mice) (Supplementary Figures 3C–F). Except for RANKL, transcription of the osteoclastogenic markers, RANK, NFATc, cathepsin K, and TRAP, was up-regulated, by 15, 3, 7, and 12 fold, respectively, in joints from mice with IL-34–induced arthritis compared to their

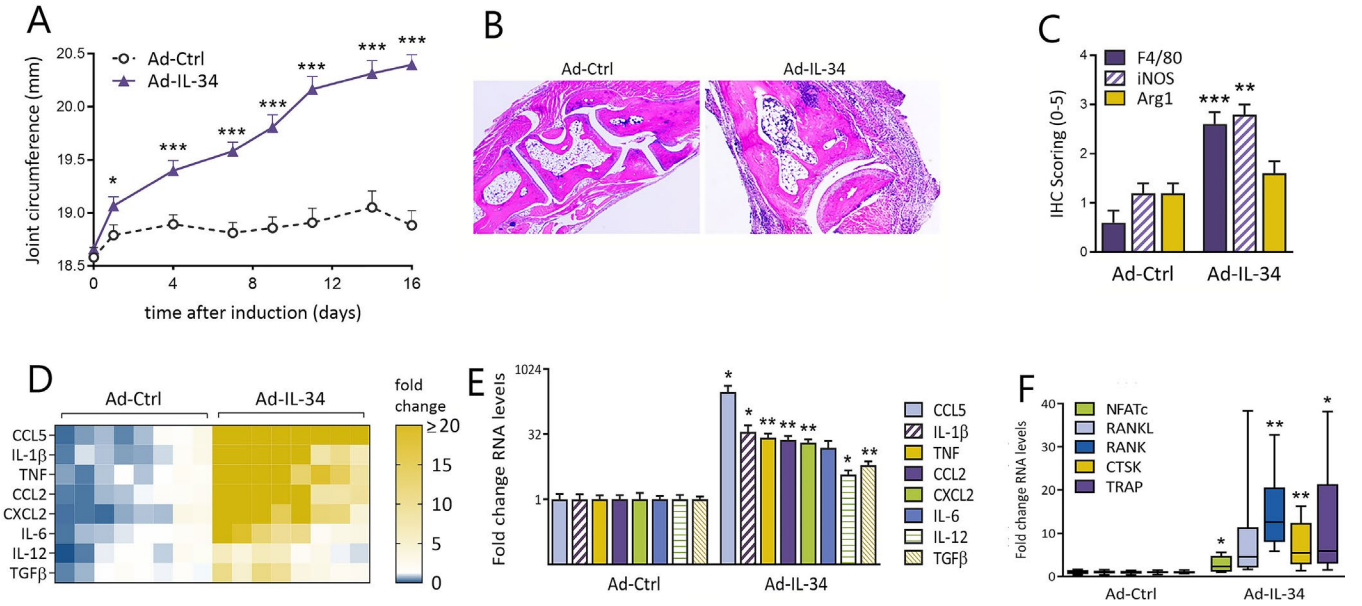

Figure 2. Induction of joint inflammation in mice by local interleukin-34 (IL-34) expression. Wild-type mice were injected intraarticularly with Ad-Ctrl or Ad-IL-34 once a week. **A**, Joint circumference was monitored over 16 days (n = 16 ankles per group). Sixteen days after arthritis onset, mice were euthanized and joints were processed for immunohistochemical (IHC) analysis, quantitative reverse transcriptase–polymerase chain reaction (qRT-PCR), and protein estimation. **B**, Tissue sections from mice treated as indicated were stained with hematoxylin and eosin and scored on a scale of 0–5 for synovial lining thickness, inflammation, and bone erosion (Supplementary Figure 3A, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41792/abstract). Representative results are shown (n = 5 ankles per group). Original magnification × 100. **C**, Tissue sections were stained for the macrophage markers F4/80, inducible nitric oxide synthase (iNOS), and arginase 1 (Arg1) (Supplementary Figure 3B), and staining was scored on a scale of 0–5 (n = 5 ankles per group). **D**–**F**, Levels of RNA for various monokines (**D** and **E**) and osteoclastic factors (**F**) in the joints of mice treated with control or Ad-IL-34 were determined by qRT-PCR (n = 8 ankles per group). Values in **A**, **C**, and **E** are the mean ± SEM. In **F**, data are shown as box plots. Each box represents the 25th to 75th percentiles. Lines inside the boxes represent the median. Lines outsie the boxes represent the minimum and maximum values. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$, versus control. TNF = tumor necrosis factor; TGFβ = transforming growth factor β; CTSK = cathepsin K; TRAP = tartrate-resistant acid phosphatase. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.41792/abstract.

control counterparts (Figure 2F). Taken together, these findings demonstrate that local IL-34 provokes arthritis, characterized by inflammatory macrophages positive for F4/80 and iNOS, and RANK^{high} osteoclasts.

Remodeling of monocytes into inflammatory M34 macrophages by IL-34 in a syndecan 1–dependent manner. Expression of IL-34 and its co-receptors in RA macrophages, as well as the amplified transcription of monokines in mice with IL-34–induced arthritis, implied that macrophages are both producers of and responders to IL-34. To delineate the mechanism of action of IL-34 in connection with syndecan 1, we examined the activation of M-CSFR and its downstream pathways. IL-34 binding triggered M-CSFR phosphorylation (tyrosine Y723), which was accompanied by the activation of the ERK and p38 MAPK pathways (Supplementary Figures 3I and J). In RA macrophages, pretreatment with syndecan 1 antibody markedly reduced M-CSFR (tyrosine Y723) and ERK phosphorylation triggered by IL-34 stimulation (Figure 3A and Supplementary Figures 3G and H). This suggests that syndecan 1 controls the initial M-CSFR activation and its downstream signaling pathways.

Naive progenitor cells stimulated with IL-34 exhibited a significant increase in CD14+CD86+ macrophage frequency (Figure 3B and Supplementary Figure 4A, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41792abstract). Consistent with these findings, while the mean fluorescence intensity (MFI) for CD14+CD206+ macrophages remained unchanged, IL-34 activation expanded the MFI for CD14+CD86+ macrophages (Supplementary Figure 4A). Reprogramming of nonactivated (M0) cells into M34 macrophages by IL-34 transformed their cytokine profile. In M34 macrophages, transcription of IL-1β, CXCL8, and CCL2 was up-regulated (3–4 fold), whereas IL-10 expression was diminished and transforming growth factor β (TGFβ) levels were unaffected (Figure 3C and Supplementary Figure 4B). Moreover, we substantiated that RA M34 macrophages secrete higher levels of CXCL8, CCL2, TNF, and IL-6 (Supplementary Figure 4C). Nevertheless, this distinct profile of M34 macrophages was disrupted by M-CSFR or syndecan 1 antibodies, as indicated by the down-regulated TNF, IL-6, and CCL2 production (up-regulation reduced by 50–70%). In conclusion, blockade of M-CSFR and syndecan 1 function reversed the M34 macrophage inflammatory profile in RA.

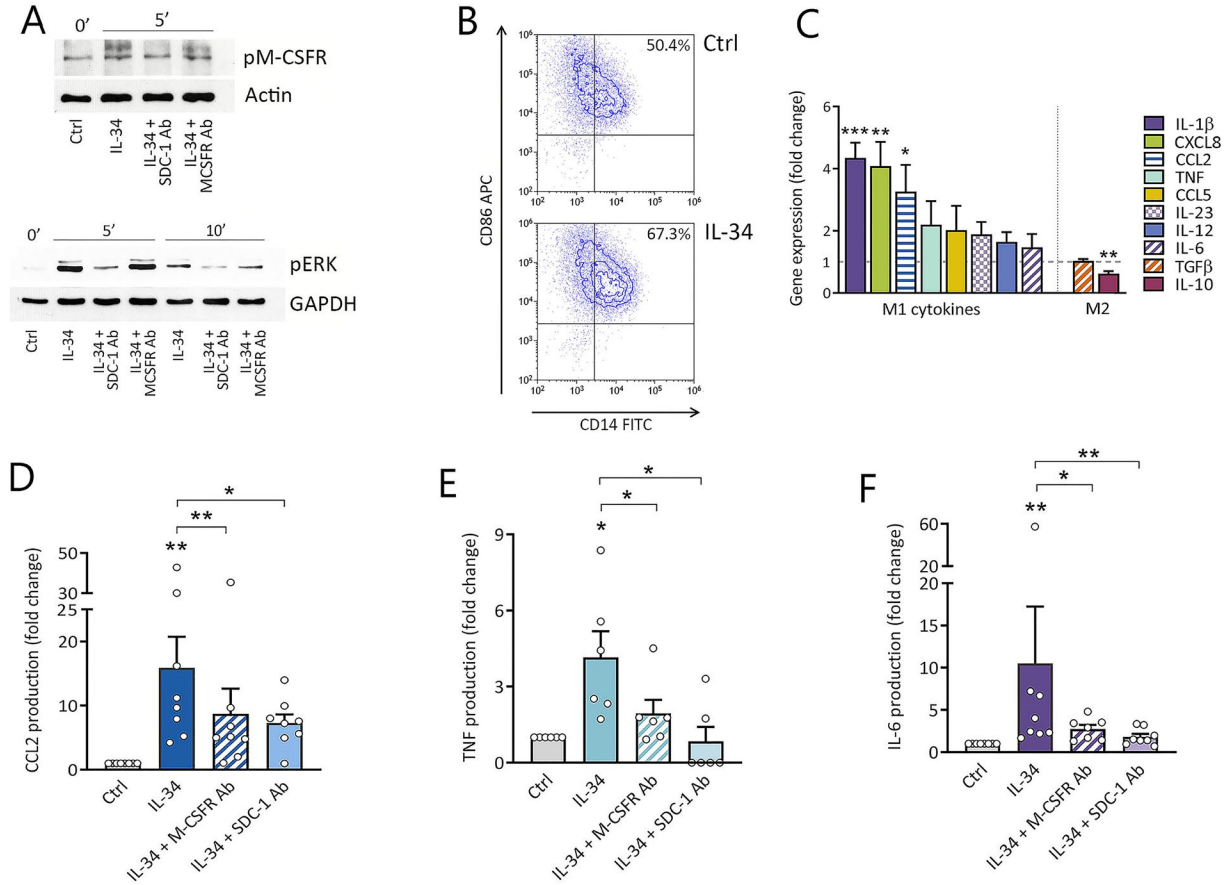

Figure 3. Dependence of IL-34–differentiated (M34) macrophage signaling and signature on syndecan 1 ligation. **A**, Cells were preincubated with buffer, anti–syndecan 1 antibody (Ab; 1:100), or anti–M-CSFR antibody (10 μg/ml) for 2 hours before stimulation with IL-34 (100 ng/ml) for 5 or 10 minutes, and lysates were used for Western blot quantification of pM-CSFR and pERK. Results are representative of 4 independent experiments. **B**, In vitro differentiated macrophages derived from negatively selected RA monocytes were treated with control or stimulated with IL-34 (300 ng/ml) for 24 hours and then stained with antibodies against CD14, CD86, and CD206 for flow cytometry. Results are representative of 5 experiments. Among CD14+ gated cells, the mean fluorescence intensity of CD86 and CD206 staining was determined (n = 5 samples per group) (Supplementary Figure 4A, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41792/abstract). **C**, Quantitative reverse transcriptase–polymerase chain reaction analysis was performed to determine the expression of M1 and M2 cytokines by in vitro differentiated RA macrophages stimulated with IL-34 (300 ng/ml) for 8 hours. Bars show the mean ± SEM fold change compared to control (n = 4 samples per group). * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$ versus control. **D**–**F**, Prior to IL-34 stimulation, RA cells were treated with control or preincubated with anti–syndecan 1 antibody (1:100) or anti–M-CSFR antibody (10 μg/ml). Induction of CCL2 (n = 8 samples per group) (**D**), tumor necrosis factor (TNF; n = 6 samples per group) (**E**), and IL-6 (n = 8 samples per group) (**F**) protein was measured. Symbols represent individual samples; bars show the mean ± SEM. * = $P < 0.05$; ** = $P < 0.01$, IL-34 versus control, by Wilcoxon's signed rank test; neutralizing antibody versus IL-34 alone, by Wilcoxon's 1-tailed matched pairs signed rank test. APC = allophycocyanin; FITC = fluorescein isothiocyanate; TGFβ = transforming growth factor β (see Figure 1 for other definitions). Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.41792/abstract.

Subsequently, we asked if IL-34 could influence other myeloid cell functions, such as phagocytosis. We found that in RA macrophages, IL-34 interfered with zymosan-triggered phagocytosis similar to the established inhibitor cytochalasin D (Supplementary Figure 4D). These results indicate that IL-34 advances the inflammatory phenotype in RA macrophages by disrupting phagocytosis.

Reprogramming of naive myeloid cells into glycolytic M34 macrophages by IL-34. RA macrophage reprogramming, instigated by IL-34, expands the population of CD14+GLUT1+ cells (Figure 4A and Supplementary Figure 4E). In addition, in murine macrophages the ECAR (which reflects glycolysis) was increased by IL-34 in a dose-dependent manner for the entire duration of the experiment (0–55 minutes) (Figure 4B). In contrast, only a brief, transient escalation of the OCR (which reflects oxidative phosphorylation) was noted in IL-34–activated murine macrophages following carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone treatment, which maximizes oxidative potential (Supplementary Figure 4F). Consistent with their increased glycolytic metabolism, IL-34–differentiated BM-derived macrophages produced higher levels of L-lactate and pyruvate (Figure 4C).

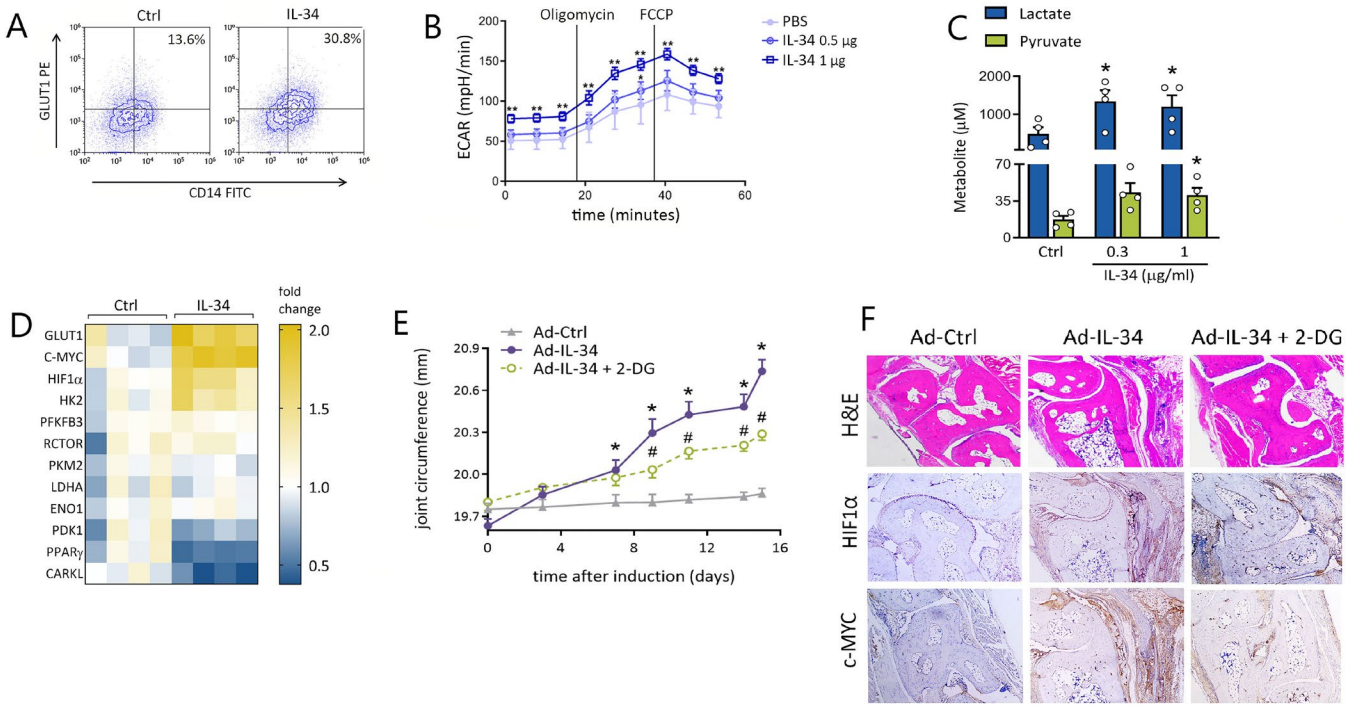

Figure 4. Promotion of progressive inflammation by interleukin-34 (IL-34)–induced metabolic changes. **A**, In vitro differentiated macrophages, derived from negatively selected monocytes, were treated with control or stimulated with IL-34 (300 ng/ml) for 24 hours. A representative contour plot is shown. Values are the mean percentage of CD14+GLUT1+ cells (n = 5 samples per group). Significance was evaluated using a paired *t*-test. **B**, A Seahorse cell energy phenotype assay was used to determine the glycolytic capacity (extracellular acidification rate [ECAR]) of phosphate buffered saline (PBS)–treated versus IL-34–pretreated RAW 264.7 cells (n = 5 samples per group). **C**, The concentration of L-lactate and pyruvate in the conditioned media of murine macrophages (treated with PBS or IL-34 for 24 hours) was determined colorimetrically. Symbols represent individual mice (n = 4 per group); bars show the mean ± SEM. **D**, Under hypoglycemic conditions, murine bone marrow–derived macrophages were left untreated or stimulated with IL-34 (1 μg/ml) for 6 hours, and the expression of metabolic genes was examined by quantitative reverse transcriptase–polymerase chain reaction (n = 4 samples per group). **E**, Wild-type mice were injected intraarticularly with Ad-Ctrl or Ad-IL-34 once per week. The mice that received Ad-IL-34 were treated intraperitoneally with either 2-deoxy-D-glucose (2-DG) or placebo, and joint circumference was monitored over 16 days (n = 10 ankles per group). **F**, Tissue sections from mice treated as indicated were stained with hematoxylin and eosin (H&E) or stained for hypoxia-inducible factor 1α (HIF-1α) or c-Myc and scored on a scale of 0–5 (n = 4 ankles per group) (Supplementary Figures 4I and J, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41792/abstract). Original magnification × 100. In **B** and **E**, values are the mean ± SEM. * = $P < 0.05$; ** = $P < 0.01$, versus control; # = $P <$ Ad-IL-34 versus Ad-IL-34 plus 2-DG. PE = phycoerythrin; FITC = fluorescein isothiocyanate; FCCP = carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.41792/abstract.

These murine M34 macrophages exhibited a skewed profile, which favored glycolytic factors over the pentose phosphate pathway or oxidative regulators, which resulted in a reduction in CARKL and peroxisome proliferator–activated receptor γ levels (Figure 4D). Accordingly, compared to nonarthritic mice, joints from mice with IL-34–induced arthritis displayed a significant up-regulation of the master modulators of glycolysis, GLUT1 (2 fold) and PFKFB3 (2 fold), as well as the glycolytic transcription factors c-Myc (3 fold) and HIF-1α (4 fold) (Supplementary Figure 4G). Since M34 macrophage polarization was linked to increased GLUT1 and glucose uptake, blood glucose concentration was down-regulated following 15 minutes and 30 minutes of glucose injection in arthritic compared to control mice (Supplementary Figure 4H).

Intriguingly, we showed that IP injection of 2-DG, an inhibitor of glycolysis, alleviates IL-34–induced joint swelling (Figure 4E). Morphologic analysis confirmed that expression of c-Myc and HIF-1α, as well as macrophages positive for F4/80 and iNOS, and CD3+ T cell infiltration, were curtailed in mice with IL-34–induced arthritis that received 2-DG therapy compared to those that received placebo (Figure 4F and Supplementary Figures 4J–L). Joint lining thickness and inflammation were similarly reversed by 2-DG treatment (Figure 4F and Supplementary Figure 4I). Taken together, our findings demonstrate for the first time that IL-34 is an endogenous factor that can reprogram RA macrophage metabolic activity.

Induction of RA FLS migration by IL-34, independently of syndecan 1 and M-CSFR. In vivo, ectopic IL-34 expression expanded joint vimentin-positive fibroblast infiltration (Supplementary Figure 5A, available on the *Arthritis & Rheumatology* website

at http://onlinelibrary.wiley.com/doi/10.1002/art.41792/abstract). In vitro, IL-34 did not significantly activate any of the signaling pathways evaluated in RA FLS, although a trend was observed for p38 phosphorylation (Supplementary Figure 5B). As previously established, RA FLS abundantly express syndecan 1 (Figure 1F). Despite the lack of full-length M-CSFR in RA FLS, IL-34 markedly activated FLS migration compared to control treatment (Supplementary Figure 5C). Surprisingly, blockade of syndecan 1 or M-CSFR function was incapable of preventing IL-34–induced RA FLS migration.

Since IL-34 has also been reported to induce M-CSFR–independent signaling through PTPRZ1, we examined the role of this proteoglycan. IL-34–mediated RA FLS migration was reduced by anti-PTPRZ1 antibody treatment (Supplementary Figure 5D), despite the rather modest expression of PTPRZ1 on RA FLS (6% PTPRZ1-positive RA FLS) (Supplementary Figure 5E). Unlike syndecan 1, PTPRZ1 expression was not elevated in RA or OA ST compared to normal ST (Supplementary Figure 5F). Due to the missing connection between PTPRZ1 and RA pathogenesis, this IL-34 study was focused on syndecan 1 and its modulation of IL-34/M-CSFR activity. In short, we found that IL-34 triggers joint as well as RA FLS recruitment independent of its primary co-receptors.

RA FLS inflammatory response and immunometabolism were not modulated by IL-34. IL-34 reprogrammed macrophages positive for syndecan 1 and M-CSFR into glycolytic and inflammatory M34 macrophages, while the transcription and/or translation of IL-6, CXCL8, CCL2, and CCL5 were unaffected by IL-34 in RA FLS (Supplementary Figures 5G and H). Corroborating these data, transcriptional regulation of GLUT1, HK2, PFKFB3, PKM2, LDHA, c-Myc, and HIF-1α was unchanged by IL-34 stimulation (Supplementary Figure 5I). Taken together, these results support the notion that the syndecan 1/M-CSFR complex is indispensable for the inflammatory and hyperglycolytic effects of IL-34, as seen in RA macrophages.

Contribution of T effector cells to IL-34–induced arthritis. IL-34 promotes Th17 cell polarization of syndecan 1–positive, M-CSFR–negative CD4+ T cells in an indirect manner (15,20,23). We found that IL-34–induced Th17 cell differentiation of murine

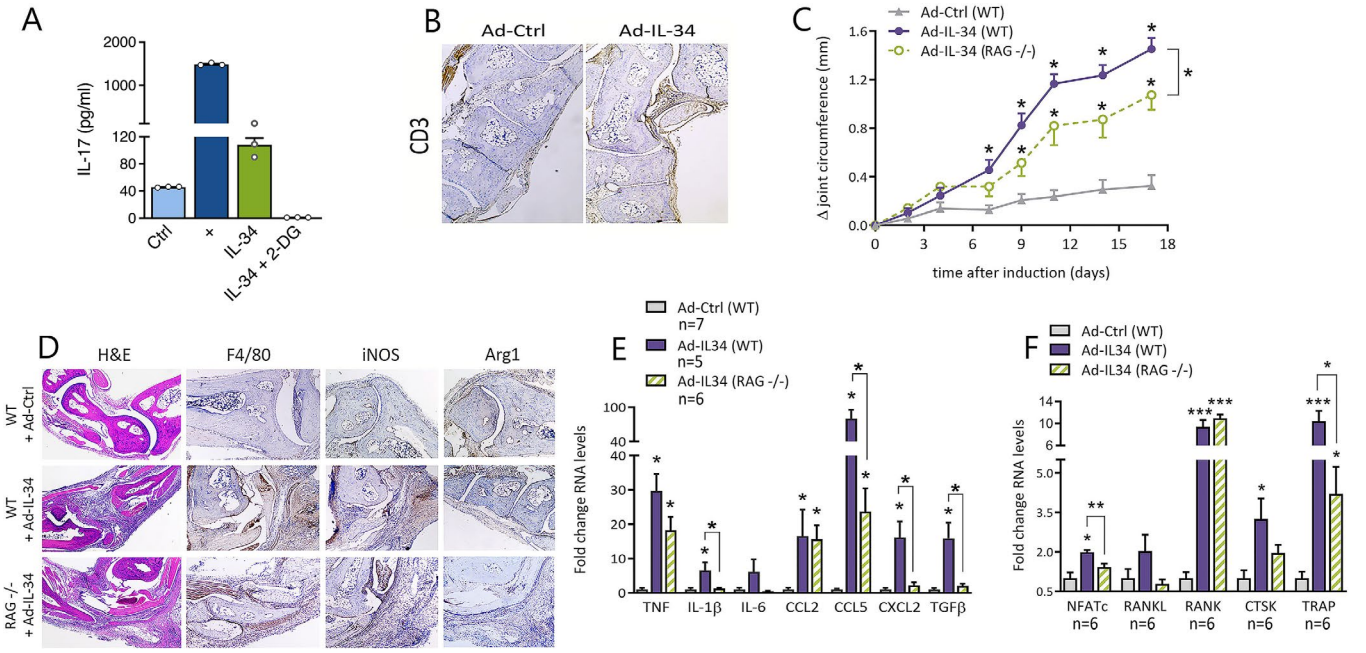

Figure 5. Contribution of T cells to IL-34–induced joint inflammation. **A**, Supernatant levels of IL-17 were measured in bone marrow–derived macrophage/splenocyte cocultures (cultured for 5 days) treated with control or IL-34 (1 μg/ml) in the presence or absence of 2-deoxy-D-glucose (2-DG; 5 m*M*). As a positive control (+) cocultures were stimulated with TGFβ (1 ng/ml) plus IL-6 (20 ng/ml). Symbols represent individual samples (n = 3 per group); bars show the mean ± SEM. **B**, Wild-type (WT) mice were treated with Ad-Ctrl or Ad-IL-34 on days 0, 7, and 14, and were euthanized on day 16. Joint CD3+ T cells were stained, and immunostaining was scored on a scale of 0–5 (n = 5 ankles per group) (Supplementary Figure 6A, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41792/abstract). Original magnification × 100. **C**, WT and RAG$^{-/-}$ mice were injected intraarticularly with Ad-Ctrl or Ad-IL-34 once per week, and joint circumference was monitored until day 17. Values are the mean ± SEM (n = 10 ankles per group). **D**, Mouse ankle joints were stained with hematoxylin and eosin (H&E) or for F4/80, iNOS, and arginase 1. H&E and positive macrophage immunostaining were scored on a scale of 0–5 (n = 4 ankles per group) (Supplementary Figures 6D and E). Original magnification × 100. **E** and **F**, Joint expression levels of monokines (**E**) and osteoclastic factors (**F**) in mice were determined by quantitative reverse transcriptase–polymerase chain reaction. Bars show the mean ± SEM. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$ versus control or as indicated. See Figure 2 for other definitions. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.41792/abstract.

splenocytes co-cultured with BM-derived macrophages requires glycolytic cell metabolism, as 2-DG treatment diminished this process (Figure 5A). In addition to macrophages, CD3+ T cell infiltration was potentiated in the ankle joints of mice with IL-34–induced arthritis (Figure 5B and Supplementary Figure 6A, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41792/abstract). Furthermore, IL-34 amplified joint Th1/Th17 cell polarization, as reflected by exacerbated interferon-γ and IL-17 production (Supplementary Figures 6B and C).

To confirm the involvement of T cells in IL-34–induced arthritis, RAG$^{-/-}$ mice were injected IA with IL-34. IL-34–induced joint swelling was mitigated in RAG$^{-/-}$ compared to wild-type (WT) mice starting on day 9 and throughout the study (Figure 5C). Morphologic analysis demonstrated that the synovial lining was similarly aggravated by local IL-34 expression in RAG$^{-/-}$ and WT mice (Figure 5D and Supplementary Figure 6D). The number of joint macrophages positive for F4/80 and iNOS was not disrupted in RAG$^{-/-}$ mice compared to WT mice that received local IL-34 administration (Figure 5D and Supplementary Figure 6E). Despite this finding, expansion of joint IL-1β, CCL5, CXCL2, and TGFβ levels (but not TNF or CCL2 levels) was reduced in RAG$^{-/-}$ mice with IL-34–induced arthritis compared to WT mice with IL-34–induced arthritis (Figure 5E).

Nonetheless, IL-34–induced bone erosion was downregulated in RAG$^{-/-}$ mice relative to WT mice, in part due to the absence of T cell–produced RANKL (Figure 5F). Loss of RANKL function in RAG$^{-/-}$ mice interfered with IL-34–induced upregulation of NFATc and TRAP but not cathepsin K (Figure 5F). Overall, IL-34–induced arthritis is exacerbated by T cell involvement, yet macrophages initiate and shape the signature IL-34–driven arthritic phenotype.

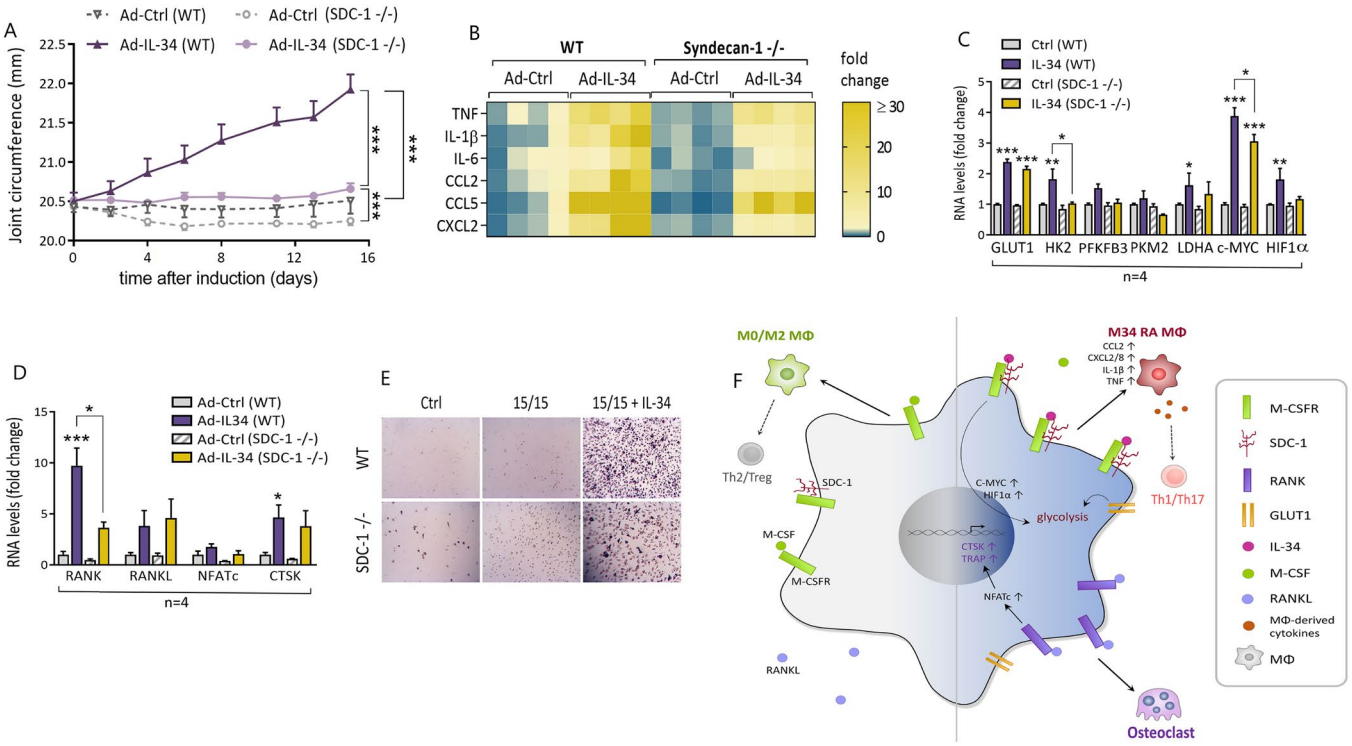

Figure 6. Modulation of interleukin-34 (IL-34)–driven joint inflammation, glycolysis, and osteoclastogenesis by syndecan 1 (SDC-1). Wild-type (WT) and SDC-1$^{-/-}$ mice were injected intraarticularly with Ad-Ctrl or Ad-IL-34 once per week. **A**, Mouse joint circumference was monitored over 15 days. Values are the mean ± SEM (n = 7 for WT mice treated with Ad-Ctrl; n = 8 for all other groups). *** = $P < 0.001$, by Mann-Whitney nonparametric test. **B**, Transcriptional regulation of joint inflammatory mediators was evaluated by quantitative reverse transcriptase–polymerase chain reaction (qRT-PCR) (n = 4 ankles per group). **C**, Under hypoglycemic conditions, bone marrow–derived macrophages from WT and SDC-1$^{-/-}$ mice were left untreated or stimulated with IL-34 (1 μg/ml) for 6 hours, and the expression of glycolytic genes was examined by qRT-PCR. **D**, Transcription levels of joint osteoclastic factors were determined by qRT-PCR in WT and SDC-1$^{-/-}$ mice that received local injection of Ad-Ctrl or Ad-IL-34. In **C** and **D**, bars show the mean ± SEM (n = 4 ankles per group). * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$ versus control or as indicated. **E**, To assess osteoclastogenesis in vitro, bone marrow–derived preosteoclasts from WT and SDC-1$^{-/-}$ mice were left untreated (Ctrl), cultured under suboptimal conditions (15 ng/ml macrophage colony-stimulating factor [M-CSF] and 15 ng/ml RANKL [15/15]), or exposed to 15/15 and IL-34 (300 ng/ml). Following 14 days of differentiation (fresh stimuli twice per week), tartrate-resistant acid phosphatase (TRAP) staining was performed and TRAP-positive multinuclear osteoclasts were counted (n = 4 ankles per group) (Supplementary Figure 6G). Original magnification × 300. **F**, IL-34 binding to M-CSF receptor (M-CSFR)/syndecan 1 cultivates rheumatoid arthritis (RA) inflammatory and glycolytic M34 macrophages (Mϕ) that are predisposed to osteoclastogenesis. HIF-1α = hypoxia-inducible factor 1α. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.41792/abstract.

Syndecan 1 defines the IL-34–induced immunostimulatory phenotype. To solidify the importance of syndecan 1 as a central modulator of IL-34–mediated disease, genetically modified mice lacking syndecan 1 were utilized (26). Progression of IL-34–induced arthritis was severely reduced in SDC-1$^{-/-}$ mice throughout the study (Figure 6A). Notably, in the absence of syndecan 1, local expression of IL-34 did provoke minor joint swelling, perhaps due to marginal M-CSFR activation. In mice with IL-34–induced arthritis, up-regulation of joint IL-1β, CCL2, CXCL2, and CCL5 levels, but not TNF levels, was impaired in SDC-1$^{-/-}$ mice compared to WT mice (reduced by 81%, 66%, 84%, and 57%, respectively) (Figure 6B and Supplementary Figure 6F).

In murine BM-derived macrophages, transcription of several components of the glycolytic pathway, including GLUT1, HK2, LDHA, c-Myc, and HIF-1α, was amplified by IL-34 stimulation (Figure 6C). Although expression of HK2 and c-Myc was suppressed, levels of GLUT1, LDHA, and HIF-1α remained unchanged in IL-34–activated SDC-1$^{-/-}$ mouse progenitor cells relative to controls (Figure 6C). Taken together, these findings indicate that the interaction between IL-34 and syndecan 1 shifts naive cells to glycolytic M34 macrophages that have a unique inflammatory and metabolic phenotype.

Requirement of syndecan 1 for osteoclasts differentiated by IL-34. In IL-34–induced arthritis, we demonstrated that the formation of TRAP-positive osteoclasts was in part due to increased RANK expression, which was disrupted in SDC-1$^{-/-}$ mice (Figure 6D). TRAP staining corroborated that IL-34–induced osteoclast formation was compromised in SDC-1$^{-/-}$ mouse progenitor cells compared to WT mice (Figure 6E and Supplementary Figure 6G). Taken together, our data indicate that syndecan 1–positive macrophages in conjunction with T cells advance IL-34–modulated osteoclastogenesis in part through RANK/RANKL function.

DISCUSSION

The present study describes a novel role for IL-34 and its co-receptor syndecan 1 in RA pathogenesis. We showed that syndecan 1 regulates myeloid IL-34/M-CSFR signaling and downstream functions. Activation of the receptor complex syndecan 1/M-CSFR is essential for the reconfiguration of naive cells to glycolytic and inflammatory CD14+CD86+GLUT1^{high} M34 macrophages. Arthritic IL-34–differentiated macrophages positive for F4/80 and iNOS are characterized by the transcriptional up-regulation of glycolytic mediators, HIF-1α and c-Myc, which are dysregulated by systemic 2-DG therapy. Intriguingly, syndecan 1 or T cell deficiency counteracts IL-34–induced joint inflammation and osteoclastic erosion in part by interfering with hyperglycolytic activity (Figure 6F).

The higher bioavailability of IL-34 compared to M-CSF in the RA SF emphasizes its ability to outcompete M-CSF for M-CSFR binding. In contrast to the shared receptor M-CSFR, the co-receptor syndecan 1, which does not bind M-CSF, is highly up-regulated in RA ST lining and sublining. Joint GM-CSF levels are also less abundant than IL-34 levels, and GM-CSF does not compete for M-CSFR or syndecan 1 binding. A growing body of evidence has demonstrated that IL-34 is involved in various pathologies, particularly in arthritis (14,34). We showed that in RA macrophages, phosphorylation of M-CSFR by IL-34 is dependent on syndecan 1. Others have shown that syndecan 1 antibody impairs IL-34–mediated monocyte migration (35). Syndecan 1 manipulates the binding affinity of IL-34 to M-CSFR (27), hence remodeling joint macrophages to glycolytic and osteoclastic phenotypes.

Syndecan 1 expression is potentiated in a range of inflammatory as well as malignant disorders (36). In cancer, elevated syndecan 1 levels are linked to exacerbated tumor size and poor prognosis. Syndecan 1 is involved in tumor invasion, proliferation, apoptosis, and angiogenesis (37). Thus, inhibitory syndecan 1 peptides and anti–syndecan 1 antibody have been generated for immunotherapy of different cancers (38). Consistent with its pathogenic implications, we show that syndecan 1 expression was highly up-regulated in RA compared to normal ST lining and sublining, attributed to macrophages, T cells, and RA FLS. Similar to RA joints, syndecan 1 is highly expressed on psoriatic arthritis ST mononuclear infiltrates, while it is barely detectable in OA ST (39). In contrast, others have suggested that syndecan 1 is involved in early-stage cartilage degeneration in experimental OA (40). It has also been shown that cannabinoid therapy attenuates OA disease activity by suppressing syndecan 1 expression (41). Nevertheless, we demonstrated that syndecan 1 up-regulation in OA relative to normal ST samples was restricted to the vasculature. A limitation of this study was that RA ST samples were obtained from de-identified patients, and therefore the expression levels of IL-34, M-CSFR, and syndecan 1 could not be linked to clinical parameters.

Our data underline that IL-34–differentiated macrophages diverge from M-CSF–induced M2 or GM-CSF–derived M1 macrophages (42). The pathogenic M34 macrophages do not rely as heavily on IL-6 or TNF, both of which are successfully targeted by current RA biologic therapies. Additionally, M34 macrophages displayed a glycolytic profile akin to that of hypermetabolic RA macrophages, yet deviating from the metabolic signature reported in lipopolysaccharide (LPS)–induced M1 macrophages. IL-34 potentiates the expression of 2 central glycolytic transcription factors, c-Myc and HIF-1α, resembling RA macrophages (43–45). However, LPS has been shown to up-regulate HIF-1α exclusively, while suppressing c-Myc transcription (46). In mice with IL-34–induced arthritis, 2-DG therapy interfered with c-Myc and HIF-1α amplification and joint swelling, highlighting that the glycolytic rewiring is linked to the IL-34–modulated inflammatory phenotype. Intriguingly, both c-Myc and HIF-1α have also been shown to instigate osteoclast formation (47). In addition to metabolic activity, RANK^{high} M34 osteoclast progenitor cells are exceptionally sensitive to T cell–derived RANKL and are prone to undergo osteoclastogenesis.

In macrophages, M-CSF, unlike GM-CSF, favors mitochondrial oxygen consumption over glycolysis, as determined by

the OCR:ECAR ratio (48). IL-34 has been suggested to induce M2 macrophage differentiation and enhance mitochondrial oxidative phosphorylation via the AMP-activated protein kinase pathway (21). In contrast, our findings identify IL-34 as a glycolytic stimulus, as evidenced by prolonged ECAR/OCR activity, accentuated pyruvate and L-lactate secretion, and transcriptional upregulation of metabolic intermediates in macrophages. Intriguingly, IL-34–driven metabolic reprogramming is expanded beyond macrophages, since 2-DG treatment negates IL-34–instigated Th17 cell polarization, suggesting that hypermetabolic activity is responsible for M34 macrophage cross-regulation with Th17 cells.

We noted that a wider range of cytokines was expressed in mice with IL-34–induced arthritis than in RA macrophages differentiated by IL-34. Perhaps Th1/Th17 cell differentiation in mice with IL-34–induced arthritis exacerbates M34 macrophage immunometabolism. Extending these findings, joint inflammation and bone erosion advanced by local IL-34 were ameliorated in T cell–deficient $RAG^{-/-}$ mice compared to WT mice due to impaired joint IL-1β, CCL5, CXCL2, and NFATc expression. In SDC-$1^{-/-}$ mice, IL-34–induced joint inflammation was alleviated through a similar mechanism as in $RAG^{-/-}$ mice (IL-1β, CCL5, CXCL2); nevertheless, osteoclastogenesis was restrained via RANK reduction. Hence, the cross-talk between the metabolic T effector cells and M34 preosteoclasts plays a critical role in IL-34–elicited inflammatory erosion. Consistent with these observations, earlier studies have illustrated that IL-34 is responsible for Th17 cell differentiation (15,20,23). Controversially, others have reported that IL-34–stimulated macrophages promote CD4+FoxP3+ Treg differentiation and thereby facilitate allograft tolerance (24).

Although TNF and PDGF promote hypermetabolic activity in RA FLS (9), neither their immunometabolic nor inflammatory profile is altered by IL-34 stimulation. In contrast to our findings, IL-6 and CXCL8 were secreted from lung fibroblasts in response to IL-34 stimulation (49). Interestingly, while IL-34 advances RA FLS migration, this function is disconnected from the inflammatory or glycolytic activity in these cells. Hence, the inability of syndecan 1 or M-CSFR blockade to negate IL-34–triggered motility suggested that RA FLS trafficking was fostered by an alternative pathway. Remarkably, IL-34 can function independently of M-CSFR or syndecan 1, through a different heparan sulfate proteoglycan, namely, PTPRZ1 (50). Modest levels of PTPRZ1 in RA FLS were shown to be responsible, at least in part, for the IL-34–mediated infiltration. However, unlike syndecan 1, PTPRZ1 is similarly expressed in RA compared to OA and normal ST samples. This is a departure from the magnified expression of PTPRZ1 on glioblastoma and colorectal cancer cells and its implication in advancing other IL-34–mediated pathologies (50,51).

Taken together, our data show for the first time that hyperglycolytic M34 macrophages and effector Th17 cells participate in inflammatory and erosive phenotypes enforced by joint IL-34 expression. While our preclinical data are promising, the therapeutic potential of IL-34 and its downstream metabolic intermediates remains to be elucidated.

ACKNOWLEDGMENTS

We want to recognize the efforts of the clinical staff of the Division of Rheumatology at The University of Illinois at Chicago, who have helped us to inform and involve patients in our studies and enabled us to pursue clinically relevant translational research. We would also like to thank Mr. Brian Zanotti (Volin Lab, Midwestern University) for technical assistance in performing the histologic studies. We thank Professor Alexander (University of Wisconsin–Madison) who kindly provided the SDC-$1^{-/-}$ mice used in this study.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Shahrara had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Van Raemdonck, Shahrara.

Acquisition of data. Van Raemdonck, Umar, Palasiewicz, Volin, Romay, Tetali, Ahmed, Zomorrodi, Shahrara.

Analysis and interpretation of data. Van Raemdonck, Umar, Palasiewicz, Elshabrawy, Amin, Sweiss, Shahrara.

REFERENCES

1. Brennan FM, McInnes IB. Evidence that cytokines play a role in rheumatoid arthritis [review]. J Clin Invest 2008;118:3537–45.
2. McInnes IB, Buckley CD, Isaacs JD. Cytokines in rheumatoid arthritis: shaping the immunological landscape [review]. Nat Rev Rheumatol 2016;12:63–8.
3. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis [review]. N Engl J Med 2011;365:2205–19.
4. Mulherin D, Fitzgerald O, Bresnihan B. Synovial tissue macrophage populations and articular damage in rheumatoid arthritis. Arthritis Rheum 1996;39:115–24.
5. Weyand CM, Goronzy JJ. Immunometabolism in early and late stages of rheumatoid arthritis [review]. Nat Rev Rheumatol 2017;13:291–301.
6. Yang Z, Shen Y, Oishi H, Matteson EL, Tian L, Goronzy JJ, et al. Restoring oxidant signaling suppresses proarthritogenic T cell effector functions in rheumatoid arthritis. Sci Transl Med 2016;8:331ra38.
7. Biniecka M, Canavan M, McGarry T, Gao W, McCormick J, Cregan S, et al. Dysregulated bioenergetics: a key regulator of joint inflammation. Ann Rheum Dis 2016;75:2192–200.
8. Garcia-Carbonell R, Divakaruni AS, Lodi A, Vicente-Suarez I, Saha A, Cheroutre H, et al. Critical role of glucose metabolism in rheumatoid arthritis fibroblast-like synoviocytes. Arthritis Rheumatol 2016;68:1614–26.
9. Falconer J, Murphy AN, Young SP, Clark AR, Tiziani S, Guma M, et al. Synovial cell metabolism and chronic inflammation in rheumatoid arthritis [review]. Arthritis Rheumatol 2018;70:984–99.
10. Geeraerts X, Bolli E, Fendt SM, van Ginderachter JA. Macrophage metabolism as therapeutic target for cancer, atherosclerosis, and obesity [review]. Front Immunol 2017;8:289.
11. Viola A, Munari F, Sanchez-Rodriguez R, Scolaro T, Castegna A. The metabolic signature of macrophage responses [review]. Front Immunol 2019;10:1462.
12. Zeisbrich M, Yanes RE, Zhang H, Watanabe R, Li Y, Brosig L, et al. Hypermetabolic macrophages in rheumatoid arthritis and coronary artery disease due to glycogen synthase kinase 3b inactivation. Ann Rheum Dis 2018;77:1053–62.

13. Yoon BR, Oh YJ, Kang SW, Lee EB, Lee WW. Role of SLC7A5 in metabolic reprogramming of human monocyte/macrophage immune responses. Front Immunol 2018;9:53.
14. Chemel M, Le Goff B, Brion R, Cozic C, Berreur M, Amiaud J, et al. Interleukin 34 expression is associated with synovitis severity in rheumatoid arthritis patients. Ann Rheum Dis 2012;71:150–4.
15. Wang B, Ma Z, Wang M, Sun X, Tang Y, Li M, et al. IL-34 upregulated Th17 production through increased IL-6 expression by rheumatoid fibroblast-like synoviocytes. Mediators Inflamm 2017;2017:1567120.
16. Rietkotter E, Bleckmann A, Bayerlova M, Menck K, Chuang HN, Wenske B, et al. Anti-CSF-1 treatment is effective to prevent carcinoma invasion induced by monocyte-derived cells but scarcely by microglia. Oncotarget 2015;6:15482–93.
17. Ge Y, Huang M, Yao YM. Immunomodulation of interleukin-34 and its potential significance as a disease biomarker and therapeutic target [review]. Int J Biol Sci 2019;15:1835–45.
18. Hamilton TA, Zhao C, Pavicic PG Jr, Datta S. Myeloid colony-stimulating factors as regulators of macrophage polarization [review]. Front Immunol 2014;5:554.
19. Huang SC, Smith AM, Everts B, Colonna M, Pearce EL, Schilling JD, et al. Metabolic reprogramming mediated by the mTORC2-IRF4 signaling axis is essential for macrophage alternative activation. Immunity 2016;45:817–30.
20. Foucher ED, Blanchard S, Preisser L, Descamps P, Ifrah N, Delneste Y, et al. IL-34- and M-CSF-induced macrophages switch memory T cells into Th17 cells via membrane IL-1α. Eur J Immunol 2015;45:1092–102.
21. Boulakirba S, Pfeifer A, Mhaidly R, Obba S, Goulard M, Schmitt T, et al. IL-34 and CSF-1 display an equivalent macrophage differentiation ability but a different polarization potential. Sci Rep 2018;8:256.
22. Liu Y, Liu H, Zhu J, Bian Z. Interleukin-34 drives macrophage polarization to the M2 phenotype in autoimmune hepatitis. Pathol Res Pract 2019;215:152493.
23. Wang B, Tang Y, Sun X, Ouyang X, Li H, Wei J, et al. Increased IL-6 expression on THP-1 by IL-34 stimulation up-regulated rheumatoid arthritis Th17 cells. Clin Rheumatol 2018;37:127–37.
24. Bezie S, Picarda E, Ossart J, Tesson L, Usal C, Renaudin K, et al. IL-34 is a Treg-specific cytokine and mediates transplant tolerance. J Clin Invest 2015;125:3952–64.
25. Baghdadi M, Umeyama Y, Hama N, Kobayashi T, Han N, Wada H, et al. Interleukin-34, a comprehensive review. J Leukoc Biol 2018;104:931–51.
26. Alexander CM, Reichsman F, Hinkes MT, Lincecum J, Becker KA, Cumberledge S, et al. Syndecan-1 is required for Wnt-1-induced mammary tumorigenesis in mice. Nat Genet 2000;25:329–32.
27. Teng YH, Aquino RS, Park PW. Molecular functions of syndecan-1 in disease [review]. Matrix Biol 2012;31:3–16.
28. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
29. Pickens SR, Chamberlain ND, Volin MV, Pope RM, Mandelin AM II, Shahrara S. Characterization of CCL19 and CCL21 in rheumatoid arthritis. Arthritis Rheum 2011;63:914–22.
30. Pickens SR, Chamberlain ND, Volin MV, Pope RM, Talarico NE, Mandelin AM II, et al. Characterization of interleukin-7 and interleukin-7 receptor in the pathogenesis of rheumatoid arthritis. Arthritis Rheum 2011;63:2884–93.
31. Kim SJ, Chang HJ, Volin MV, Umar S, Van Raemdonck K, Chevalier A, et al. Macrophages are the primary effector cells in IL-7-induced arthritis. Cell Mol Immunol 2020;17:728–40.
32. Umar S, Palasiewicz K, Van Raemdonck K, Volin MV, Romay B, Amin MA, et al. IRAK4 inhibition: a promising strategy for treating RA joint inflammation and bone erosion. Cell Mol Immunol 2020. doi: 10.1038/s41423-020-0433-8. E-pub ahead of print.
33. Van Raemdonck K, Umar S, Palasiewicz K, Volkov S, Volin MV, Arami S, et al. CCL21/CCR7 signaling in macrophages promotes joint inflammation and Th17-mediated osteoclast formation in rheumatoid arthritis. Cell Mol Life Sci 2020;77:1387–99.
34. Chang SH, Choi BY, Choi J, Yoo JJ, Ha YJ, Cho HJ, et al. Baseline serum interleukin-34 levels independently predict radiographic progression in patients with rheumatoid arthritis. Rheumatol Int 2015;35:71–9.
35. Segaliny AI, Brion R, Mortier E, Maillasson M, Cherel M, Jacques Y, et al. Syndecan-1 regulates the biological activities of interleukin-34. Biochim Biophys Acta 2015;1853:1010–21.
36. Fears CY, Woods A. The role of syndecans in disease and wound healing [review]. Matrix Biol 2006;25:443–56.
37. Palaiologou M, Delladetsima I, Tiniakos D. CD138 (syndecan-1) expression in health and disease [review]. Histol Histopathol 2014;29:177–89.
38. Rousseau C, Ferrer L, Supiot S, Bardies M, Davodeau F, Faivre-Chauvet A, et al. Dosimetry results suggest feasibility of radioimmunotherapy using anti-CD138 (B-B4) antibody in multiple myeloma patients. Tumour Biol 2012;33:679–88.
39. Patterson AM, Cartwright A, David G, Fitzgerald O, Bresnihan B, Ashton BA, et al. Differential expression of syndecans and glypicans in chronically inflamed synovium. Ann Rheum Dis 2008;67:592–601.
40. Salminen-Mankonen H, Saamanen AM, Jalkanen M, Vuorio E, Pirila L. Syndecan-1 expression is upregulated in degenerating articular cartilage in a transgenic mouse model for osteoarthritis. Scand J Rheumatol 2005;34:469–74.
41. Kong Y, Wang W, Zhang C, Wu Y, Liu Y, Zhou X. Cannabinoid WIN55,2122 mesylate inhibits ADAMTS4 activity in human osteoarthritic articular chondrocytes by inhibiting expression of syndecan1. Mol Med Rep 2016;13:4569–76.
42. Ushach I, Zlotnik A. Biological role of granulocyte macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) on cells of the myeloid lineage [review]. J Leukoc Biol 2016;100:481–9.
43. Asahara H, Hasunuma T, Kobata T, Inoue H, Muller-Ladner U, Gay S, et al. In situ expression of protooncogenes and Fas/Fas ligand in rheumatoid arthritis synovium. J Rheumatol 1997;24:430–5.
44. Pap T, Nawrath M, Heinrich J, Bosse M, Baier A, Hummel KM, et al. Cooperation of Ras- and c-Myc–dependent pathways in regulating the growth and invasiveness of synovial fibroblasts in rheumatoid arthritis. Arthritis Rheum 2004;50:2794–802.
45. Roivainen A, Soderstrom KO, Pirila L, Aro H, Kortekangas P, Merilahti-Palo R, et al. Oncoprotein expression in human synovial tissue: an immunohistochemical study of different types of arthritis. Br J Rheumatol 1996;35:933–42.
46. Liu L, Lu Y, Martinez J, Bi Y, Lian G, Wang T, et al. Proinflammatory signal suppresses proliferation and shifts macrophage metabolism from Myc-dependent to HIF1α-dependent. Proc Natl Acad Sci U S A 2016;113:1564–9.
47. Park-Min KH. Metabolic reprogramming in osteoclasts [review]. Semin Immunopathol 2019;41:565–72.
48. Izquierdo E, Cuevas VD, Fernandez-Arroyo S, Riera-Borrull M, Orta-Zavalza E, Joven J, et al. Reshaping of human macrophage polarization through modulation of glucose catabolic pathways. J Immunol 2015;195:2442–51.
49. Zhou J, Sun X, Zhang J, Yang Y, Chen D, Cao J. IL-34 regulates IL-6 and IL-8 production in human lung fibroblasts via MAPK, PI3K-Akt, JAK and NF-κB signaling pathways. Int Immunopharmacol 2018;61:119–25.
50. Nandi S, Cioce M, Yeung YG, Nieves E, Tesfa L, Lin H, et al. Receptor-type protein-tyrosine phosphatase ζ is a functional receptor for interleukin-34. J Biol Chem 2013;288:21972–86.
51. Franze E, Dinallo V, Rizzo A, Di Giovangiulio M, Bevivino G, Stolfi C, et al. Interleukin-34 sustains pro-tumorigenic signals in colon cancer tissue. Oncotarget 2018;9:3432–45.