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Macrophage M1/M2 polarization and rheumatoid arthritis: A systematic review

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ABSTRACT

Background and aim: Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease; the clinical manifestations are correlated with continuum multiarticular synovitis, cartilage and bone damage, and defeat of joint function, that causes disability. Involvement of internal organs is also frequent. Between the inflammatory cells involved in RA, macrophages play a key role. These cells can polarize in different phenotype and mediate

Abbreviations: RA, Rheumatoid Arthritis; M1, classically activated macrophages; M2, alternatively activate anti-inflammatory macrophages; AP, Cs, antigen presenting cells; TNF-α, tumoral necrosis factor- alpha; IL, interleukin; TGF-β, transforming growth factor- beta; PRISMA, Preferred Reporting Items for Systematic reviews and Meta-Analyses; WOS, Web of Science; SD, Science Direct; PB, peripheral blood; FACS, fluorescence-activated cell sorting; CD, Cluster Differentiation; HD, healthy donors; JIA, Juvenile Idiopathic Arthritis; SJIA, systemic-JIA; IFN-γ, interferon-gamma; MDM, Monocytes derived macrophages; GM-CSF, Granulocyte/Monocytes-Colony Stimulation Factor; M-CSF, Macrophages-Colony Stimulation Factor; MFI, mean fluorescence intensity; OC, osteoclast; OA, osteoarthritis; MΦ, polarized macrophages; FR-β, Folate Receptor beta; iNOS, inducible nitric oxide synthetase; PHD3, prolyl hydroxylase 3; CCR, C-C motif chemokine receptor; MMP, matrix metalloproteinase; EGLN3, egl nine homolog 3; INHBA, inhibin subunit beta A; CypA, Cyclophilin-A; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; TSPO, translocator protein; LPS, lipopolysaccharide; SIRT, Sirtuin; AMPK, adenosine monophosphate-activated protein kinase; RSV, Resveratrol; MDC, macrophage-derived chemokine; FcεRII, Fc epsilon RII; MrC1, C-type mannose receptor 1; SEMA3A, Semaphorin 3A; DAS28, Disease Activity Score 28; RF, Rheumatoid Factor; PBMC, peripheral blood mononuclear cells; BMM, Bone Marrow Macrophages; ROS, reactive oxygen species; ARG1, arginase1; Fizz1, found in inflammatory zone 1; Ym1, mouse chitinase-like 3; μRNA, micro-Ribonucleic Acid; CCL, (C-C motif) chemokine ligand; ACPAs, Anti-citrullinated protein antibodies; ESR, Erythrocyte sedimentation rate; CRP, C-reactive protein; TLRs, Toll Like-Receptors; IRF, contain two proteins involved in Toll Like-Receptors (TLRs) signaling, namely Interferon; bDMARDs, biological disease-modifying antirheumatic drugs; MerTK, Tyrosine protein kinase Mer; SOCS3, Suppressors of cytokine signaling 3; Gas6, MerTK ligand; pSTAT3, phospho Signal Transducer and activator of transcription; Pam3, Pam3CysSerLys4; FOLR2, FR-β 2; SLC40A1, solute carrier family 40 member 1; HMOX1, heme oxygenase 1; STIA, sterile arthritis; IVIG, intravenous gamma-globulin; AIA, antigen-induced arthritis; FcγRI, Fc gamma receptor I; IL-1RII, (IL)-1 receptor II; SIO, steroid induced osteonecrosis; CIA, collagen induce arthritis; IL-$10^{-/-}$, IL-10 null mice; $IRF5^{-/-}$, (IRF)-5 null mice; Tg, transgenic; WT, wild type; 11β-HSD, 11 beta-hydroxysteroid dehydrogenase; GCs, glucocorticoids; Tg197, mouse model that express stabilized human TNF-α; KO, knock out; CSF1, colony stimulating factor 1; Hes1-GFP, Drosophila hairy and enhancer of split 1 plus green fluorescence protein; CH3, C3H/HeNCrl; B6, C57BL/6NCr; Bbaa1, Borrelia burgdorferi–associated locus 1; IFN, interferon; MFC-MSNS, Manganese ferrite and ceria nanoparticle-anchored mesoporous silica; ML-WA, Withaferin-A incorporated in mannosylated liposomes; Pae, paeoniflorin; CP-25, Paeoniflorin-6′-O-benzene sulfonate; AA, adjuvant induced arthritis; MTX, Methotrexate; AP, alkaline phosphatase; sSiglec-9, sialic acid-binding immunoglobulin-type lectin; p65, NF-kb subunit/cofactor proto-oncogene RelA; FUTs, fucosyltransferases; 2-D-gal, fucose/galactose analog; siRNA, silencing RNA; CB2, Cannabinoid Receptor 2; JWH133, 3-(1′1′Dimethylbutyl)-1-deoxy-D8-tetrahydrocannabinol CB2 agonist; CXCR3, C-X-C motif chemokine receptor 3; JN2, (N-(4-(5-chlorobenzo[*d*]oxazol-2-ylamino)phenyl) – 4-aminobutanamide) CXCR3 antagonist; CXCL, C-X-C motif chemokine ligand; RANKL, TNF superfamily member 11; Tnfsf11, RANKL transcript; TCR, T cells receptor; KRN, mouse strain that stably express TCR; NOD, non-adaptive immunologic function; SCID, severe combined immunodeficiency; $IL2R\gamma^{-/-}$, (IL)-2R gamma null; M0, unpolarized macrophages; DMSO, dimethyl sulfoxide; MHCII, major histocompatibility complex II; ON, osteonecrosis; FLS, Fibroblast like synovitis; ET-1, endothelin-1; ESR, erythrocyte sedimentation rate; ETA, etanercept; ADA, adalimumab; CTLA4-Ig, Cytotoxic T-Lymphocyte Antigen 4- Immunoglobulin; IHC, immunohistochemical

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the immune/inflammatory reaction as well as the reparatory phase when possible. The properties of these cells are mediate by the body's environmental factors.

In this systematic review, all English-speaking articles concerning the role of M1 (pro-inflammatory) or M2 (anti-inflammatory) macrophages in RA were systematically reviewed and categorized according to their polarized-function in RA, especially in the synovial tissue. Analyses of the endogenous molecules and the drugs that could modulate M1 and M2 activity in RA were achieved.

Methods: A sensitive search was developed in Pubmed, Web of Science, Ovid Med-Line, Embase Database and Science Direct Database (la both from Elsevier) to identify articles to increase the highlighting on the role of macrophages M1 and M2 in RA using the following terms: ((M1 AND M2) AND Rheumatoid Arthritis). All selected papers were read and discussed by two independent reviewers. The selection process was based on title, abstract and full text level. Relevant data were extracted and analyzed using a standardized template designed for this review.

Results: In total 39 resulting articles were selected and categorized according to description of M1/M2's role in RA. Data from humans, mice and rats were subcategorized, thus in every section were highlighted the contribute, in peripheral blood and synovial tissue, of both polarized macrophages; section for endogenous molecules and drugs that favor the switch from M1 to M2 macrophages were carried out. The most evinced relevant results, were that in RA blood and in the synovial tissue, there isn't a clear distinction phase with M1 or M2 macrophages (by membrane marker analysis); rather there is M1 and M2 subset disequilibrium and by deeply analyses of mRNA gene and cytokine produced, it emerged that a non-coherent expression inner marker match with membrane molecules, and also the tissue section can define the marker expressed.

Conclusion: This systematic review emphasizes that the rigid classical subdivision of M1 and M2 macrophages, as well as the different samples' results comparison, might be questionable. In addition, it is suggested, when taking samples from RA patients, to carefully consider their therapies in order to analyze the M1 and M2 macrophages behavior without drug influence.

In line with the advances in M1 and M2 knowledge, and the progression in the single-cell methodologies by identification of individual cell molecular markers, therapeutic approaches seem possible to favor the anti-inflammatory macrophage response in RA (e.g. M2 polarization).

1. Introduction

Rheumatoid Arthritis (RA) is a chronic immune-mediated inflammatory disease that impacts almost 1% of the population worldwide. It causes advanced joint damage and mainly affects the synovial tissues, cartilage and bone [1]. Furthermore, the systemic and organ complications of RA are associated with a high incidence of morbidity and rising mortality [2,3]. Over the last fifty years, scientific contribution has produced numerous concepts as to how immune responses impact the RA disease [3].

This immune-mediated disorder involves, both innate and adaptive cellular compartments as well as their dysregulated cytokine production, moreover the cellular resident in the bone and in joint compartments, such as osteoclast, chondrocyte and stromal cells are involved [4]. Within the innate immunity function that causes inflammation, macrophages have a key role. In fact, they contribute to the normal tissue homeostasis, working as APCs to activate adaptive immunity [5,6], pathogen expulsion, resolution of inflammation and wound healing.

In particular, the studies relating to the function of macrophages in inflammation have progressed gradually and scientists have identified two different polarization states [4,7].

Classically activated macrophages (M1), causes joint erosion, secreting principally proinflammatory cytokines such as TNF-α and IL-1 and alternatively activate macrophages (M2), which through a high production of anti-inflammatory cytokines (mainly IL-10 and TGF-β) contributes to vasculogenesis, tissue remodeling and repair, as recently observed in systemic sclerosis. Markers for both phenotypes may coexists on the same cell as recently reported for the first time in systemic sclerosis by our group [8,9].

The aim of this systematic review, using Med-Line and non-Med-Line research, is to summarize the previously published data on M1/M2's role in RA synovial tissue, as well as discussing selected molecules/drugs in animal models and in RA patients that might modulate M1 activities and facilitate M2 phenotype activation with tissue damage reduction and/or repair.

2. Methods

2.1. Search strategy

A systematic search, in line with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA [10]) guidelines, was carried out on Pubmed, WOS, Ovid (Med-Line database), Embase and SD (Elsevier Databases). It was performed without any limitation to the publication date in order to identify articles focused on the role of M1 and M2 macrophages cellular compartments in the RA disease (evaluated in Humans, Mice, Rats) in particular, highlighting their role in synovial tissue.

The Medical terms for RA were used in combination with M1 and M2 (note that we used the “AND” Boolean Logic Symbol, to restrict the area of investigation, as follow: M1 AND M2 AND Rheumatoid Arthritis). The structured search was last updated on 3th May 2019.

2.2. Screening process

All articles were screened by two independent reviewers (ST and GM). The reviewing selection process was based on title, abstract and full-text level, using Covidence [11,12], a tool, produced in partnership with Cochrane Reviews which simplify the systematic review process. All studies, explaining the activities of M1 and M2 macrophages in the pathogenesis of RA, were included in the systematic review with particular inclusion of articles which focused on synovial tissue.

All study designs, with the exception of reviews, editorials, case studies and conference abstracts/posters, were included. Languages, other than English, were excluded, as well as studies that were not available in full-text version format. However, titles and abstracts selected by either one of the reviewers were included for additional screening. At each level of the screening process, where different opinions existed among the ST and GM as to whether to include a record or not, it should be noted that a mutual agreement was reached (see Fig. 1. for the flowchart).

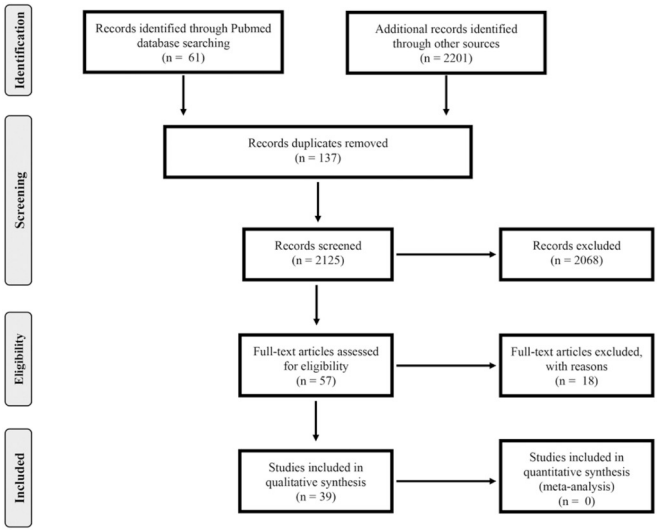

Fig. 1. Data are given as number of selected articles categorized according to four different parts of the search process: identification, screening, eligibility and inclusion. The format of this figure is based on PRISMA 2009 guideline [10].

In addition, all selected full-texts meeting the inclusion criteria were once again, checked by ST and GM. In the same way, relevant data was extracted and analyzed using a standardized template designed for this review.

3. Results

Of the 2262 articles screened (by title, abstract and full-text level) that were considered potentially relevant to the topic of this study, 39 articles were included. The flow of the searching and selection process is shown in Fig. 1. Supplementary File 1 summarizes all 39 selected manuscripts focused on M1 (classically-activated macrophages) and M2 (alternatively-activated macrophages) in RA. The 39 selected articles (the detailed data are shown in Table 1) were categorized according to the description of M1/M2's role in RA: the data corresponding to human and rodents (i.e. mice and rats) have been considered as the two main categories since they have an impact on M1/M2 cellular compartments. Within these two groups, the records evaluated were those from macrophage polarization contribution on inflammation or tissue repair, endogenous molecules that are important in maintaining macrophages homeostasis or that exacerbate the pro-inflammatory macrophages response, together with exogenous molecules (drugs) that impact and can modulate the M1/M2 reaction. All records considered macrophage activity, especially in peripheral blood and synovial tissue.

3.1. Human

Macrophages play an important role in RA. Inflamed RA synovial tissue and inflammatory vascular reactions both presented a higher number of macrophages compared to healthy joints [13,14].

In inflammatory conditions, macrophages were found to be widespread in the RA lining of the synovial tissue [15]. The circulating monocytes represent the principal source of these cells. These PB monocytes exist in three different populations which are grouped based on their expression of cell-surface molecules and functions [16]. Phenotypical FACS analysis of PB classic $CD14^{+}CD16^{-}$, intermediate $CD14^{2+}CD16^{+}$ and non-classic $CD14^{dim}CD16^{+}$ monocytes reveal no cellular membrane marker difference between RA patients and HD [17].

Moreover, studies in Juvenile Idiopathic Arthritis (JIA) or Systemic-JIA (SJIA), common forms of arthritis in children and adolescents, confirmed that the distribution of the $CD14^{+}$ and $CD16^{+}$ sub-populations are not altered in comparison to HD [18].

3.1.1. M1 and M2 in peripheral blood from rheumatoid arthritis patients

Studies of both ex-vivo and in vitro PB monocyte markers, which simulate the polarization of M1 or M2 macrophages, have been carried out in the past few years.

$CD14^{+}$ monocytes were purified from the PB of RA patients and compared with the same cells from HD in order to evaluate if ex-vivo and in vitro polarization markers of M1 and M2 macrophage cellular compartments are already appreciable. Conversely, CD64 ($M\Phi$-$_{IFN\text{-}\gamma}$), CD-200R ($M\Phi$-$_{IL\text{-}4}$) and CD-16 ($M\Phi$-$_{IL\text{-}10}$) labeling did not show a significant difference between the two groups [17]. Monocytes-derived macrophages (MDM) stimulated with GM-CSF or M-CSF in vitro, did not show any particular difference in M1 or M2 marker expressions (GM-CSF M1 macrophage express CD163 and CD206 which should be M2 markers) and the same results were obtained from ex-vivo monocytes analysis (evaluation of monocytes % and/or MFI) [19].

Confirmation of these results comes from another study where scientists analyzed M1 ($CD68^{+}CD192^{+}$) and M2 ($CX3CR1^{+}CD163^{+}$), and, once again, no significant difference was found, concluding that RA PBs seem to be composed by mixed M1 and M2 monocyte sub-populations [19,20].

Likewise, in SJIA, PB surface phenotyping monocytes compared to HD did not show any significant difference; spontaneous cytokine production analysis confirmed the same result [18].

Nevertheless, a more in-depth analysis among M1 and M2 markers under flare and quiescence conditions resulted in a more robust difference. In fact, $CD14^{+}$ monocytes showed mixed M1/M2 at flare and instead an antigen panel towards M2-like pic at quiescence. However, $CD16^{+}$ monocytes did not change their surface markers during flare or quiescence and were seen to be more associated with M1-like marker production.

Furthermore, Fukui et al. confirmed that the distribution of monocyte sub-types, divided by CD14 and CD16 expression, showed no significant differences between RA patients and HD. In addition, M1/M2 correlated in a significantly-positive manner with the OC number [21].

3.1.2. M1 and M2 from rheumatoid arthritis synovial tissue

Macrophages in inflamed synovial tissue are the cells which are mainly activated in RA and are the principal source of cytokines, such as TNF-α, which contribute to the disease progression. If indeed macrophages play a pathogenic role in RA, as confirmed by numerous experimental data, the degree of joint erosion and the contribution to hyperplasia of the intimal synovial lining layer could also be linked to the increased number in the synovial tissue [22].

It should be noted that there is a macrophage subset disequilibrium from RA patients' synovial fluid with the M1/M2 ratios being higher in RA compared to OA patients [21,23]. In a double-immunofluorescence staining study, the $CD68^{+}$ marker has been colocalized with the following markers: of IFN-γ dependent polarization markers ($M\Phi_{IFN\text{-}\gamma}$), of IL-4 dependent polarized markers ($M\Phi_{IL\text{-}4}$), of IL-10 dependent polarized markers ($M\Phi_{IL\text{-}10}$) [17,24].

Intimal lining layer was found to contain mainly mature resident macrophages (the $M\Phi_{IL\text{-}10}$ markers CD163 and CD32 colocalized with $CD68^{+}$), whereas the synovial sublining showed more mixed phenotypes ($CD68^{+}$ colocalized with $M\Phi_{IL\text{-}10}$ markers CD163 and CD32 and the $M\Phi_{IFN\text{-}\gamma}$ marker CD64, and the $M\Phi_{IL\text{-}4}$ CD200R and $CD14^{+}$), suggesting that it is actively infiltrated with immature MDM. Moreover, disease activity in RA seemed associated with the number of synovial sublining macrophages, but not with intimal lining layer macrophages [17]. In addition, $CD163^{+}$ synovial-derived macrophages colocalize with the FR-β and this expression depends on their distribution in synovial sub-tissue.

In fact, FR-β is more expressed by $CD163^{+}$ in the sublining layer of the synovial tissue and these cells produce more iNOS, and more IL-10

Table 1
Detailed description of selected articles' results.

Main text number	Authors	Study type	Sample	Type of administered drug	Species (Subject/Animal)	Study question	Main result
#16	Ambarus et al. - Arthritis Research & Therapy 2012, 14:R74	IHC, FACS, Double IF	Blood, Synovial Biopsies	Patients therapeutic information: None Patients were treated with bDMARDs; No other therapeutic Patients information.	Human - 11 Spa and 8 RA Patients, 9 HD for PBMC samples; 18 Spa & 20 RA for IHC analysis	Polarization status of Macrophages in SpA and RA	* No difference in monocytes subsets in Blood samples, RA Vs HD. * No difference in MDM polarized RA Vs HD. * Cd163 (M2) markers up-regulated in IHC analysis Spa Vs RA * RA intimal lining layer contains mainly mature resident macrophages * RA synovial sublining is actively infiltrated with immature MDM (same in RA and SpA).
#45	Degboe Y. Et al. - Front. Immunol. 2019;10:3	FACS, WB, qRT, ELISA, Pharmacological inhibition	Blood	Patients therapeutic information: bDMARDs, no GC < 10 mg, no Steroid or intraarticular injection of steroids	Human - 20 Ra patients; 30 HD	in vitro modulation of macrophage polarization by bDMARDs targeting pro-inflammatory cytokines in RA	* Anti-TNF agent induce change in macrophages polarization status. * Anti-TNF agent induce down-regulation of surface markers M1 like CD40 and CD80. * Anti-TNF agent induce up-regulation of surface marker M2 like CD16, CD163, MerTK). * Anti-TNF agent inhibit inflammatory cytokines (TNF, IL-6, IL-12) * Anti-TNF agent increase phagocytosis (via IL-10 and elevated level contributions of Gas6, SOCS3 and pSTAT3). * Other bDMARDs, did not induce any significant polarization in macrophages subsets.
#20	Fukui S. et al. - Front. Immunol. 2018; 8:1958.	FACS, ELISA	Blood	Patients therapeutic information: bDMARDs, concomitant use with MTX or Prednisolone	Human - 40 RA Patients and 20 HD subjects	Relationships among M1 monocytes, M2 monocytes, osteoclast (OC) differentiation ability, and clinical characteristics in patients with rheumatoid arthritis (RA).	* M1/M2 ratio correlates with the number of OCs in the RA patients. * M1/M2 ratio is the sole responsible of the up-regulation of the OCs number. * ACPA+, RF and ESR value not are co-contributing factors to the numbers of the OCs. * RA patients with M1/M2 ratios > 1 (having relatively more M1 monocytes) had higher CRP and ESR than RA patients with M1/M2 ratios ≤ 1.
#32	Narayan N. et al. - PLoS ONE. 2017; 12(10): e0185767	IF, qRT, ELISA, Radioligand Saturation assay, WB	Synovial Tissue, vitro, RNA	Patients therapeutic information: No therapeutic Patients information	Human - 5 RA Patients	Investigation of TSPO expression in human monocyte derived macrophages (MDM), and if there is a contribution in macrophage polarization	* TSPO magnitude up-regulation was greater in RA synovial fluids' MDM, than blood from HD. * TSPO mRNA expression is reduced in M1 macrophages. * TSPO transcript is down-regulated after LPS and IFN-γ treatment in MDM stimulated than unstimulated MDM. * TSPO reduction start after 24 h hours of treatment LPS and IFN-y in synovial fluid.

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Table 1 (*continued*)

Main text number	Authors	Study type	Sample	Type of administered drug	Species (Subject/Animal)	Study question	Main result
							* TSPO expression did not show any significant difference in M2 MDM than unstimulated MDM.
#33	Park SY. et al. - Front. Immunol. 2017; 8:1135.	qRT, WB, RNA-interference, IHC, in vivo experiment	Synovial Tissue, RNA	Patients therapeutic information: No therapeutic Patients information - Arthritic induction: CII + CFA + CII + IFA. Other administration: RSV 50 and/or Sirtinol.	Human RA Patients, SIRT1-Tg CIA Mice model of Arthritis	Investigation of potential immunomodulatory effect of (SIRT)-1 in modulation of M1/M2 in RA	Human derived tissue: * SIRT1 activation induce anti-inflammatory M2 macrophages and attenuates the expression of pro-inflammatory M1 macrophages. * SIRT1/ AMPKα is the signaling pathway that leads to anti-inflammatory function of macrophages in RA. * SIRT1 prevents NF-κB p65 acetylation/phosphorylation, resulting in reduced M1 polarization. Mice derived tissue: * SIRT1-Tg CIA mice developed less severe arthritis than WT CIA mice
#18	Quero L. et al. - Arthritis Research & Therapy. 2017; 19(1):245	FACS, qRT, WB, ELISA	Blood Sample, RNA	Patients therapeutic information: No therapeutic Patients information	Human RA Patients	Investigation of the impact of TLR stimulation on M1 and M2 macrophage function and phenotype	* TLR2 stimulation (but not TLR4) ligands drives M2-polarized macrophages to secrete pro-inflammatory cytokines and impairs their anti-inflammatory activity. * TLR2 stimulation induce generation of chimeric M1/M2 macrophage subset without major changes in the surface marker profile. * They postulate that the classical M1/M2 paradigm as based on surface marker expression does not apply to macrophage functions under inflammatory conditions such as RA.
#38	Schulert GS. et al. - Arthritis & Rheumatology. 2016; 68(9):2300–13	Microarray, qRT, silencing miRNA	Blood Sample, Vitro assay, RNA	Patients therapeutic information: No therapeutic Patients information	Human - 27 JIA Patients, 15 HC	Determination of the expression profiles of monocytes from children with systemic JIA. Expression of microRNA-125a-5p and its contribution to monocyte polarization	* MicroRNA-125a-5p (of 110 mRNAs found) was identified as being highly up-regulated in monocytes from children with active systemic JIA. * Monocyte miR125a-5p expression was increased after polarization under M2b or M2c conditions. * miR-125a-5p over-expression enhanced M2b polarization and altered other polarized populations Vitro. * miR-125a-5p over-expression altered the macrophage phenotype towards the same phenotype observed in systemic JIA.
#24	Tsuneyoshi Y. et al. - Scand J Rheumatol 2012; 41:132–40	FACS, IF, IHC	Synovial Tissue	Patients therapeutic information: Prednisolone 5 mg	Human - 15 OA Patients, 12 RA Patients	Distribution of FR-ββ macrophages and their	* FR-β macrophages were distributed predominantly in the sublining layer of RA synovial tissues.

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Table 1 (*continued*)

Main text number	Authors	Study type	Sample	Type of administered drug	Species (Subject/Animal)	Study question	Main result
						polarization in OA and RA synovial tissues.	* RA synovial FR-β or CD163 macrophages express a mixed pattern of M1 and M2 macrophages markers. * Findings underscore that the M1/M2 paradigm using surface markers FR-β and CD163 is an oversimplification of macrophage subsets.
#72	Vandooren B. et al. - Arthritis & Rheumatism. 2009; 60(4):966–75	FACS, ELISA	Synovial Fluid, Blood Sample	Patients therapeutic information: NSAIDs, Corticosteroids, bDMARDs.	Human - 55 RA, 47 Spa, 15 PsA Patients	Investigation of: if differential infiltration with macrophage subsets was associated with a different local inflammatory milieu in SpA as compared with RA	* RA SF inhibited the expression of CD200R and CD163, even in the presence of the M2-polarizing factor IL-10. * SF levels of M1-derived mediators correlated well with peripheral joint inflammation in RA.
#19	Zhao J.Z. et al. - APMIS. 2017; 125(12):1070–75.	FACS	Blood Sample	Patients therapeutic information: NSAIDs, bDMARDs.	Human - 120 AS, 55 RA Patients; 100 HD	Evaluation of the polarization of peripheral blood monocytes in the patients with AS compared to RA; and to determine the correlations between monocyte polarization and inflammation and structural damage	* RA show a mixed monocyte subset (M1 and M2). * RA's M1 (CD68 + CD192+) polarized monocytes seemed to be predominant (than M2 CXC3R1 + CD163+) and correlated with ESR and CRP.
#22	Zhu W. et al. - Inflammation. 2015; 38(6):2067–75	ELISA, FACS, WB, IHC	Synovial Fluid, Blood Sample	Patients therapeutic information: No therapeutic Patients information	Human - 39 RA; 28 OA Patients - IRF5−/− Mice model to study Arthritis	Investigation in samples from rheumatoid arthritis (RA) patients to examine whether ACPAs alter macrophage subset distribution and promote RA development	* Macrophage subset disequilibrium occurred in RA patient SF. * IRF4 and IRF5 protein expressions were detected in SF. * IRF5 in under control of ACPAs activity and lead to M1 macrophage polarization.
#30	Dongsheng Z. - Inflammation. 2017; 40(5):1761–72	Histology, ELISA, FACS, qRT, WB, Knockdown or Overexpression experiment	Synovial Tissue (Mice & Humans), Serum, RNA	Patients therapeutic information: no patients included was being treated with corticosteroids. Arthritic induction: CII, CFA + IFA.	Human 15 RA, 15 OA patients - CIA Mice model of Arthritis	Explore the effects of Cyclophilin A (CypA) on macrophage polarization and describe the underlying mechanisms.	* CypA induced augmentation level of pro-inflammatory cytokines, (IL-6, IL-1β, and IL-17). * CypA induce increase in number of pro-inflammatory M1 macrophages in synovial fluid. * The percentage of M1 pro-inflammatory macrophage was increased than the percentage M2 anti-inflammatory macrophage that was decrease. * Increasing of pro-inflammatory M1 macrophages are the responsible of the arthritis aggravation observed in CypA.
#65	Li J. et al. - Arthritis & Rheumatology. 2014; 66(9):2368–79	FACS, Cell Sorting, Histology, in vivo study, Knock-out study	Synovial fluid and Tissue, Serum Samples	Patients therapeutic information: Methotrexate or TNF inhibitor (Enbrel or Remicade). Arthritic induction:	Human 14 Ra; 14 OA Patients, − CIA Mice model of Arthritis	Determination of the roles of fucosylation in rheumatoid arthritis (RA) and to assess the efficacy of reestablishing immune	* FUTs that are involved in terminal or subterminal fucosylation. * FUTs are up-regulated in RA compared to OA synovial tissue.

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Table 1 (*continued*)

Main text number	Authors	Study type	Sample	Type of administered drug	Species (Subject/Animal)	Study question	Main result
				CII + CFA + CII + IFA; Other administration: 2-D-gal, fucose.		homeostasis with the use of 2-deoxy-D-galactose (2-D-gal), a fucosylation inhibitor.	* Terminal FUTs predominantly expressed in M1 macrophages. * In vivo, 2-D-gal treatment of mice precluded the development of CIA by reducing inflammatory M1macrophages. * In vitro, treatment with 2-D-gal skewed the differentiation of M1 macrophages to IL-10–producing M2 macrophages. * 2-D-gal significantly inhibited the antigen-presenting function of M1 macrophages.
#64	Matsumoto et al. - Arthritis Research & Therapy. 2016; 18(1):133	IHC, FACS, qRT, ELISA, Biofluore scence Imaging	Ankle Joint, Synovial Tissue, Vitro cellular protein Lysate, RNA	Arthritic Induction: Bovine CII + CFA + BovineCII + IFA. Other administration: sSiglec-9 at a dose of 5 ng/g. 50 μM RS504393. Patients therapeutic information: No therapeutic Patients information	5 Human RA Patients - DBA/1 J strain models for Arthritic induction	The aim of this study was to assess the effects sSiglec-9 on joint inflammation and destruction in a murine collagen-induced arthritis (CIA) model and FLS derived from patients with rheumatoid arthritis.	* sSiglec-9 significantly suppressed the clinical and histological incidence and severity of arthritis. * sSiglec-9 reduced the expression of M1 markers in macrophages, it did not affect the expression of M2 markers. * Nuclear factor (NF)-kB p65 phosphorylation was attenuated by sSiglec-9. * Chemical blockade of the NF-kB pathway reduced M1 marker expression.
#36	Teng Y. et al. - Oncotarget, 2017;8(39) 66270–80	ELISA, qRT, WB, Histology	Peripheral Blood (PB), Synovial Tissue, RNA	Arthritis Induction: serum from arthritic K/BxN; Other administration: Sema3a or GFP cDNA inserted into the pAd/CMV/V5-DEST Gateway® Vector - Patients medication: Aminoglucose, or Sodium hyaluronate, or NSAID, or TCM, or DMARDs, or Glucocorticoid.	Human - 20 RA; 20 OA Patients, 10 HD - Mice STA model of arthritis	Is Sema3A a diagnostic maker and therapeutic avenue for RA?	* Sema3a correlated negatively with the levels and RA severity. * Sema3A promoted IL-4 induced M2 macrophage polarization. * Sema3A prohibited LPS/IFN-γ induced M1 polarization. * Sema3A retarded osteoclastogenesis. * In vivo: Sema3A administration attenuated joint tissue damage and the severity of experimental arthritis.
#41	Kang K. et al. - Immunity. 2017. 47.235–50.e4	qRT, RNA-sequencing, Gene Ontology Analysis, Chromatin Immunoprecipitation, ChIP-seq Library Preparation and Sequencing, Assay for Transposase-Accessible Chromatin using Sequencing, ChIP-seq and ATAC-seq Analysis, Distribution Plot of ChIP-seq and ATAC-seq Signals, Motif Identification, Motif Enrichment, Super-Enhancers, Functional,	Synovial macrophages, RNA	Patients Therapeutic information: Dexamethasone	9 RA Patients, 5 HD	Investigation of the mechanisms by which IFN-g represses gene expression and the functions of such genes. We chose to examine IFN-g-mediated repression in primary human macrophages given the central role of these cells in human inflammatory diseases, including rheumatoid arthritis (RA).	* IFN-γ suppresses genes important for homeostatic M2 like. * The repressed by IFN-γ GC-inducible genes were refractory to induction by the GC dexamethasone. * The DEX genes expression decrease of was associated with deactivation of enhancers. * IFN-γ-repressed enhancers were enriched for binding sites for the transcription factor MAF. * MAF and its M2-like target genes were suppressed in RA synovial macrophages, revealing a disease-associated signature of IFN-γ-mediated repression.

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Table 1 (*continued*)

Main text number	Authors	Study type	Sample	Type of administered drug	Species (Subject/Animal)	Study question	Main result
		Adenoviral Transduction, Analysis of Gene Expression in RA Synovial Macrophages					* IFN-γ disassembles enhancers to suppress expression of M2-related homeostatic and reparative genes. * MAF is an important regulator of the macrophage enhancer landscape and an M2-gene-expression mediator that is targeted by IFN-γ to augment macrophage activation.
#17	Macaubas C. et al. - Clinical Immunology (2012) 142, 362–72	ELISA, qRT, FACS,	RNA, Blood	Patients Therapeutic information: No therapeutic Patients information	14 SJIA Patients	Characterization of the activation state (classical/M1 vs. alternative/M2) and of the immunophenotype of SJIA monocytes, using several approaches.	* CD14 + +CD16− and CD14 + CD16+ monocyte subsets are activated in SJIA. * A mixed M1/M2 activation phenotype is apparent at the single cell level, especially during flare. * Consistent with an M2 phenotype, SJIA monocytes produce IL-1β after LPS exposure, but do not secrete it. * Despite the inflammatory nature of active SJIA, circulating monocytes demonstrate significant anti-inflammatory features.
#39	Li D. et al. - Molecular Immunology 2017;87:329–30. Corrigendum.	FACS, WB, qRT, ELISA	Blood	No information about patients' therapies	SJIA Patients (Clinically Active and Inactive Disease)	Determination of the expression of miR-146a in freshly isolated CD14+ monocytes from children with SJIA, and to investigate the effects of miR-146a in monocyte polarization.	* miR-146a expression was elevated in patients with active SJIA. * miR-146a may be involved in the alternative activation of M2 macrophage phenotype (Both in vivo and in vitro). * miR-146a is a negative regulator of M1 polarization. * miR-146a contributes to the generation of alternatively activated M2 macrophages.
#37	Woo W. Et al. - EBioMedicine 2018;38,228–37	FACS, qRT, WB	Mice Synovial Tissue, Mice BMM, RNA	Arthritis induction: CII + CFA + CII + IFA + l-ipopolysaccharide (LPS, 5 μg/100 ml); K/BxN serum for induction of arthritis.	Human - 20 RA (7 severe RA, 13 Mild RA), 23 OA Patients -Mice - C57BL/6 mice strain of mS6KO for Arthritis induction.	Investigation of the clinical implication of myeloid Sirt6 in RA	* mRNA levels of M1 macrophage-related genes, chemokines and their receptors, and protease were increased, while M2 macrophage marker genes were decreased. * Sirt6-deficient macrophages have an increased potential for migration towards joint synovium in part via CCR3 upregulation through a yet unidentified mechanism. * The protein levels of Sirt6 were markedly decreased in synovial tissues, blood-derived monocytes, and synovial fluid monocytes of RA patients compared with OA patients. * The enzymatic activity of Sirt6 were closely related to the inflammatory activity of RA patients.
#21	Palacios B.S. et al. - J Pathol 2015; 235: 515–26	FACS, IHC, qRT, WB	Synovial Tissue	Patients Therapies: Anti-TNF, Anti-CD20, MTX,		Examination of the molecular basis for the	

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Table 1 (*continued*)

Main text number	Authors	Study type	Sample	Type of administered drug	Species (Subject/Animal)	Study question	Main result
				leflunomide Drugs, gold salts.	Human - 44 Patients with active RA - 6 Patients with inactive	tissue-damaging effects of macrophages in RA joints,	* Synovial fluid from RA Patients shows high amount of production of pro-inflammatory gene and protein. * Synovial Membrane macrophage labeled with CD163+ produce high level of pro-inflammatory cytokines. * Activin-A M1 macrophage and inhibits M2 macrophage
#50	Hofkens W. et al. - PLoS One. 2013; 8(2):e54016.	Luminex multianalyte technology, FACS, microarray, qRT.	Synovial Tissue, Macrophages Superna-tant, RNA	Arthritis Induction: mBSA + CFA + mBSA + IFA + mBSA + saline. Other administration: prednisolone disodium phosphate for liposomal PLP); Liposomal-PLP or free PLP; ICA: lysozyme + saline + anti-lysozyme antibodies.	Human - AIA or ICA, C57Bl/6 mice strain for Arthritic induction	Determination of the effect of the liposomally delivered glucocorticoid prednisolone phosphate (Lip- PLP) on M1/M2 polarization of macrophages within the synovial intima layer.	* Human Microarray analysis of biopsies of inflamed synovium (AIA mice) showed an increased M1 signature characterized by upregulation of IL-1β, IL-6 and FcγRI. * Lip-PLP strongly suppressed joint swelling and synovial infiltration. Colloidal gold containing liposomes exclusively targeted the macrophages within the inflamed synovial intima layer. Vitro studies. * Lip-PLP phagocytosed by M1 macrophages resulted in a suppression of the M1 phenotype and induction of M2 markers (IL-10, TGF-β, IL-1RII, CD163, CD206 and Ym1). Vivo studies. * Lip-PLP treatment strongly suppressed M1 markers (TNF-α, IL-1β, IL-6, IL-12p40, iNOS, FcγRI and CD86) after local M1 activation of lining macrophages with LPS and IFN-g and during experimental AIA and immune complex arthritis (ICA)
#63	Chandrupatla D.M.S.H. et al. - Transl Res. 2018; 199:24–38.	Histopathology & IHC, IF, Ex vivo studies, PET scanning	Rats' knee	Arthritic induction: ripetute injection of mBSA	Wistar Rats strain to induction of Arthritis	Examination of AP's broad mechanism of action constitutes a safe therapeutic for RA	* AP considerably down-modulate the synovial tissue macrophage infiltration (both M1 and M2 subsets); these results are comparable with MTX administration alone. * AP and MTX injection combination had synergic effects. * AP also revealed systemic anti-inflammatory effects in reduction of ED1 (CD68+), ED2 (CD163+), and FRβ + macrophages of arthritic rats. * AP, just like MTX, may thus impact synovial infiltration of polarized inflammatory macrophages.
#59	Kim J et al. ACSNano.2019 13(3):3206–17	IHC, qRT, H&E staining,	Synovial Tissue, RNA	Arthritic Induction: CFA. Other administration: MFC-MSNs, C-MSNs, MF-MSNs, and MFC-MTX.	AIA Rats model of Arthritis	Investigation of manganese ferrite and ceria nanoparticle-anchored mesoporous silica	* MFC-MSNs exhibit a synergistic effect on O2 generation and ROS scavenging.

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Table 1 (*continued*)

Main text number	Authors	Study type	Sample	Type of administered drug	Species (Subject/Animal)	Study question	Main result
						nanoparticles (MFC-MSNs) that can synergistically scavenge ROS and produce O2 for reducing M1 macrophage levels and inducing M2 macrophages for RA treatment.	* MFC-MSNs lead to efficient polarization of M1 to M2 macrophages both in vitro and in vivo.
#60	Jain S. et al. - Biomaterials. 2015; 61: 162–77	qRT, MRI, ELISA, Histopathology	Synovial Tissue, RNA, in Vivo Imaging, Serum	Arthritic induction: killed mycobacterium butyricum + IFA	AIA Rats model of Arthritis	Investigation if non-viral gene transfection strategy can re-polarize macrophages from M1 to M2 functional sub-type for the treatment of rheumatoid arthritis (RA).	* In arthritic rats the intensity of the M1 marker (CD86) was significantly higher (80% total macrophages) than that of the M2 marker (CD163–9% of the total macrophages). * IL-10 delivery with tuftsin-modified nanoparticles loaded with IL-10 plasmid DNA increase in M2 phenotype (66%). * Moreover the levels of pro-inflammatory cytokines were significantly reduced (ELISA, qRT result).
#58	Ma Y. et al.- J Immunol 2014; 193(12):6050–60	In vivo study, qRT, Phagocytosis assay	Serum and joint tissue sample, RNA	Lyme Arthritis induction: Borrelia burgdorferi infection	(CH3, B6, K/BxN) Mice models - B6-IFNAR1 −/−, CH3-IFNAR1 −/− Lyme Arthritis models	B. burgdorferi–associated locus 1 (Bbaa1) associated to Lyme Arthritis. Utilization of forward genetic analysis to identify quantitative trait loci (QTLs) controlling the difference in Lyme arthritis severity in B6 and C3H mice. How Bbaa1 controlling severity of Lyme arthritis and RA.	* The Bbaa1 locus modulated the functional phenotype ascribed to bone marrow–derived macrophages. * The B6 allele promoted expression of M2 markers. * The C3H allele promoted induction of M1 responses. * Identification of Bbaa1 as a regulator of type I IFN during both Lyme arthritis and RA development. * Inherent patterns of gene expression, regulated by Bbaa1 alleles, direct the functional phenotype of macrophages as classically (M1) versus alternatively (M2) polarized.
#49	Misharin et al. - Cell Reports. 2014;9: 591–604	FACS, qRT, Microarray	Blood, Synovial Joint Sample, RNA	75 μl of arthritogenic serum	K/BxN Mice arthritis model	Characterization of the different subsets and/or polarized phenotypes of monocytes and macrophages that may play distinct roles during the development and resolution of RA inflammation.	* ✵Nonclassical Ly6C-monocytes are required for the initiation and progression of sterile joint inflammation. * ✵Nonclassical Ly6C- monocytes differentiate into inflammatory macrophages (M1), which drive disease pathogenesis and display plasticity during the resolution phase. * During the development of arthritis, these cells polarize towards an alternatively activated phenotype (M2), promoting the resolution of joint inflammation. * All of these steps occur without changes in synovial tissue-resident macrophages.

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Main text number	Authors	Study type	Sample	Type of administered drug	Species (Subject/Animal)	Study question	Main result
#61	Sultana F. et al. - Colloids and Surfaces B: Biointerfaces. 2017; 155:349–365	qRT, WB, ELISA, Confocal Study.	Synovial Tissue, RNA, Serum	Arthritis induction: CFA + heat-killed *Mycobacterium tuberculosis*. Other administration: Free-WA, L-WA, ML- WA; ML-DP.	AIA Rats arthritis model	Evaluation of if the delivery of ML-WA ameliorates inflammation and bone resorption in adjuvant-induced arthritic rats through the modulation of oxidative stress, osteoclastogenic imbalance (RANKL and OPG), and macrophage repolarization from M1 (pro-inflammatory) to M2 (anti-inflammatory) phenotype.	* ML-WA ameliorated the severity of inflammation and bone resorption in AIA rats. * ML-WA down-regulates pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, MCP-1 and VEGF). * ML-WA increase the production of IL-10; down-regulates the level of CD86 mRNA, while it amplifies mRNA of CD163.
#54	Sun W. et al. - Journal of Bone and Mineral Research. 2017; 32(7):1469–80	FACS, IF, ICC,	Synovial fluid and tissue	Drugs/molecules administration: retrovirus for 3 days + M-CSF + THAP	Hes1-GFP/TNF-Tg double-transgenic mice arthritis model	Verification of that RA synovium promotes Notch activation in bone marrow–derived macs, resulting in their M1 polarization, and Notch inhibition attenuates joint tissue damage by reducing M1 macs.	* Synovial M1 macrophages come from bone marrow, and become Notch-active in response to the joint microenvironment. * They are also more predisposed to M1 polarization. * Thapsigargin (THAP), a Notch inhibitor, reduces TNF-induced M1 mac formation and promotes a M2 mac phenotype via Notch
#64	Wang Q. et al. - Biomaterials 2017; 122:10–22.	FACS, confocal exp., FRET, BCA assay, Silencing exp. (siRNA), qRT, ICC	Serum, RNA	Arthritis Induction: bovine CII + CFA + bovine CII + CFA. Other administration: P65 (RelA) siRNA, DEX, LPS, BSA.	CIA Mice model of arthritis	Examination of the ability of micelles loaded with Dex and siRNA to inhibit NF-kB signaling in macrophages as well as switch macrophages in the arthritic synovium from the M1 to M2 state. Evaluation of the therapeutic efficacy and safety in a mouse model of collagen-induced arthritis	* Hybrid micelles loaded with Dex and siRNA effectively inhibited NF-kB signaling in murine macrophages. * The co-delivery system was able to switch macrophages from the M1 to M2 state.
#55	Ye L. et al. - Arthritis Research & Therapy 2014;16:R96	Confocal exp., FACS, IF, ELISA, WB, Histopathology	Serum and Synovial fluid sample	Arthritis induction: CII, CFA + Mycobacterium Tuberculosis	CIA Mice IL10−/− model of arthritis	Determination of the signaling pathway of interleukin 10 (IL-10) for modulating IL-17 expression in macrophages and the importance of this mediation in collagen-induced arthritis (CIA).	* Compared to WT mice, IL-$10^{-/-}$ mice had exacerbated CIA development. * IL-10 deficiency might promote macrophage polarization towards the pro-inflammatory M1 phenotype, which contributes to the rheumatoid arthritis inflammation response.
#67	Zhu M. et al. - J Bone Miner Res. 2019;34:739–51	Histopathology, IHC, IF, ELISA, WB, qRT	Serum of PB, RNA	Arthritis Induction: CII + CFA + heat killed mycobacterium tuberculosis + CII + IFA; Other administration: CB2 agonist JWH133; CB2 antagonist SR144528.	CIA Mice model of arthritis	Verification the effect of CB2 on macrophage polarization during the inflammation of CIA	* JWH133 treatment decreased the infiltration of pro-inflammatory M1-like macrophages. * JWH133 repolarized macrophages from the M1 to M2 phenotype. * activation of CB2 increased the expression of anti-inflammatory cytokine interleukin (IL)-10 and reduced the expression of pro-

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Main text number	Authors	Study type	Sample	Type of administered drug	Species (Subject/Animal)	Study question	Main result
							inflammatory cytokines TNF-α, IL-1β, and IL-6. * JWH133 inhibited RANKL-induced NF-κB activation in the osteoclast precursors.
#52	Hardy R.S. et al. - Journal of Autoimmunity 92 (2018) 104–13	FACS, qRT, Histology, ELISA	Knee joint, Synovial tissue Sample, RNA	NO administration of Exogenous Drugs	Tg197 mice (TNF-Tg) model of arthritis; (11β-HSD1 KO - TNF-tg11βKO) mice models of arthritis.	Investigation the consequences of global and mesenchymal specific 11β-HSD1 deletion in the Tg197 (TNF-Tg) murine model of chronic poly- arthritis	* TNF-tg11βKO mice marked increase in M1 macrophages than TNF-Tg mice. * TNF-tg11βKO mice marked skewing of the M1/M2 ratio favoring M1 polarization. * 11β-HSD1 protected bone from inflammation-associated bone loss.
#66	Kim B. et al. - European Journal of Pharmacology 823 (2018) 1–10	ELISA, qRT, WB, Histology	CIA Mice BMMs, RNA, Serum	Arthritis Induction: CII + CFA + heat killed Mycobacterium tuberculosis + CII + IFA. Other administration: JN-2 or DMSO.	CIA Mice arthritis model	Evaluation of the effectiveness of JN-2, our newly developed CXCR3 antagonist, in an animal model of arthritis and elucidation of the molecular mechanisms of action.	* The increased levels of TNF-α, IL-6, and RANKL in mouse serum following CIA were greatly inhibited in JN-2-injected mice (same in vitro). * CIA-induced mRNA expression of TNF-α, IL-6, and Tnfsf11 was inhibited by JN-2-injected mice. * JN-2 inhibited CXCR3-induced cell migration and cytokine production by blocking the cascade of CXCR3 signaling, leading to the amelioration of arthritis progression. * JN-2 Indirectly decrease of M1 Macrophages production molecules and mRNA.
#62	Chang Y. et al. - Sci Rep. 2016 May 17; 6:26239	ELISA, qRT, FACS, Histology	Synovial Tissue, Ankle joint, RNA	Arthritis induction: heat-killed Mycobacterium butyricum + CFA. Other administration: CP-25, TGP, or Pae or MTX.	AA - Lewis Rats arthritis model	Evaluation of the potential anti-arthritic activity of CP-25, in in rats with adjuvant-induced arthritis (AA).	* CP-25 inhibited the production of pro-inflammatory cytokines TNF-α, IL-1β, IL- 6 and IL-17. (vitro, serum). * CP-25 increased the TGF-β1 production. (vitro, serum). * The effects of CP-25 was similar to the effects of MTX. * CP-25 treatment inhibited RANKL production and altered the RANKL/OPG ratio in favor of anti-osteoclastogenic activity.
#69	Elisia I. et al. PLoS One. 2016 Mar 31;11(3):e0152538	ELISA, qRT, FACS	Synovial Tissue, PB, RNA	Arthritis induction: K/BxN arthritogenic Serum. Other administration: DMSO	K/BxN Mouse model of Arthritis	Evaluation of the DMSO as an anti-inflammatory agent using a novel whole human blood assay designed to measure the ability of blood cells, ex vivo, to respond to a bacterial and viral challenge.	* The fore paws of the DMSO-treated mice than the hind paw and mice control, expressed significantly ($P < .05$) lower levels of pro-inflammatory genes (IL-1β, IL-6, CXCL1 and CXCL2). * From BM Macrophages of non-Arthritic mice DMSO appears to block the polarization of M0 macrophages to either M1 or M2.

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Table 1 (*continued*)

Main text number	Authors	Study type	Sample	Type of administered drug	Species (Subject/Animal)	Study question	Main result
#56	Weiss M. et al. - Mediators Inflamm. 2013: 245804	qRT, Wb, ELISA, FACS	Knee joint tissue, BMM (Naive Mice)	Arthritis Induction: ripetute injection of mBSA in CFA + mBSA in PBS	AIA, C57Bl/6 mice strain for Arthritic induction.	Characterization of the murine macrophages and IRF5 expression in both in vitro and in vivo models of inflammation	* Pro-inflammatory macrophages do express higher levels of IRF5 than CD206+ macrophages (vitro, vivo). * Analysis of whole knee RNA extracts supported these observations and demonstrated that IRF5 transcript levels are significantly augmented in affected knees. * Amount of pro-inflammatory macrophages at the site of inflammation correlate with an increase in IRF5 mRNA and protein.
#51	Wu X. et al. - International Journal of Immunopathology and Pharmacology, 351–61.	Histology, FACS, IHC, ELISA, qRT.	Synovial Tissue, PB, Serum, RNA.	ON induction: methylprednisolone	BALB/C mice for ON induction	Investigation of the roles of macrophages in the progression of steroid-induced osteonecrosis, with an emphasis on the role of TNF-a; and to explore the mechanisms for their activation and polarization.	* In the early stage of ON, there was high TNF-α activity. * Large population of M1 macrophages infiltrated into the necrotic zone. * On the contrary, the expression of TNF-α gradually decreased. * Correlated to TNF-α decreasing, augmented larger M2 cell population presented in the necrotic zone in the late stage of ON. * TNF-α mediated alteration of M1/M2 macrophage polarization contributed to the pathogenesis of steroid-induced osteonecrosis.

IHC (Immunohistochemical), FACS (Fluorescence Advanced Cell Sorting), IF(Immunofluorescence), WB (Western Blot), qRT (quantitative Real-Time), ELISA (Enzyme-Linked Immunosorbent Assay), RNA (Ribonucleic Acid), miRNA (microRNA), PET (Positron-Emission Tomography), H&E (Hematoxylin & Eosin), MRI (Magnetic-Resonance-Imaging), ICC (immunocytochemical), FRET (Fluorescence Resonance Energy Transfer), BCA (bicinchoninic acid assay), siRNA (silencing Ribonucleic Acid), ChIP-seq (Chromatin Immunoprecipitation Sequencing), ATAC-Seq (Accessible Chromatin with High-Throughput sequencing), RA (Rheumatoid Arthritis), PB (Peripheral Blood), CIA (Collagen Induce Arthritis), BMM (Bone Marrow-Macrophages), bDMARDs (biological Disease-Modifying Antirheumatic Drugs), GC (Glucocorticoid), mg (milligrams), MTX (Methotrexate), MDM (Monocyte Derived Macrophage), RSV (Resveratrol), μM (micromolar), mBSA (methylated Bovine Serum Albumin), ml (milliliters), CII (bovine/chicken type II collagen), CFA (Freund's Complete Adjuvant), IFA (Freund's incomplete adjuvant), Kg (Kilogram), 2-D-gal (2-deoxy-D-galactose), BW (Body Weight), MFC-MSNs (Manganese Ferrite and Ceria-Mesoporous Silica Nanoparticles), C-MSNs (Ceria-Mesoporous Silica Nanoparticles), MF-MSNs (Manganese Ferrite - Mesoporous Silica Nanoparticles), MFC-MTX (Manganese Ferrite and Ceria-Methotrexate), sSiglec-9 (soluble Sialic acid-binding immunoglobulin-type lectin), RS504393 (C-C chemokine receptor type 2, CCR2 antagonist), WA (Withaferin-A), L-WA (Liposomal-WA), ML-WA (mannosylated-liposomal-WA), ML-DP (mannosylated-liposomal - Dexamethasone palmitate), M-CSF (Macrophages Colony stimulating factor), THAP (Thapsigargin), K/BxN Mice (Mice expressing the transgenic T cell receptor (TCR) KRN and the MHC class II allele), NSAID (nonsteroidal anti- inflammatory drug), TCM (Traditional Chinese Medicine), DMARDs (disease-modifying antirheumatic drugs), SemA3A (Semaphorin3A), GFP (GreenFluorescentProtein), pAd/CMV/V5-DEST (Adenoviral plasmid with Cytomegalovirus promoter V5 purification Tag and ATG codon for mammalian protein expression), p65 (NF-kB protein cofactor RelA), DEX (Dexamethasone), LPS (Lipopolysaccharide), BSA (bovine serum albumin), CB2 (Cannabinoid Receptor II), JWH133 (3-(1,1-Dimethylbutyl)-1-deoxy-Δ8-THC, 3-(1,1-Dimethylbutyl)-1-deoxy-Δ8-tetrahydrocannabinol), SR144528 (5-(4-Chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-N-[(1*S*,2*S*,4*R*)-1,3,3-trimethylbicyclo [2.2.1]hept-2-yl]-1H-pyrazole-3-carboxamide), JN-2 (N-(4-(5-chlorobenzo[*d*]oxazol-2-ylamino) phenyl) − 4-aminobutanamide), DMSO (Dimethyl sulfoxide), CP-25 (Paeoniflorin-6′-O-benzene sulfonate), TGP (total glucosides of peony), PAE (monoterpene glucoside paeoniflorin), mBSA (methylatedBSA), ICA (Immunocomplex Arthritis), PLP (Prednisolone disodium phosphate), PBS (Phosphate-buffered saline), SIRT1-Tg (Sirtuin 1 Transgenic - stabilized expression), Spa (Spondyloarthritis), RA (Rheumatoid Arthritis), HD (Healthy Donor), PBMC (Peripheral Blood Mononuclear Cell), JIA (Juvenile Idiopathic Arthritis), OA (Osteoarthritis), PsA (Psoriatic Arthritis), AS (Ankylosing Spondylitis), CIA (Collagen Induced Arthritis), AIA (Adjuvant Induced Arthritis), CH3 (C3H/HeNCr), B6 (C57BL/6NCr), CH3-IFNAR1$^{-/-}$ (C3H/HeNCr-Deficiency for Interferon alpha receptor 1), B6-IFNAR1 −/− (C57BL/6NCr-Deficiency for Interferon alpha/beta receptor subunit 1), Hes1-GFP/TNF-Tg (hes family bHLH transcription factor 1-GFP/Tumoral Necrosis Factor Transgenic mRNA), STA (Serum transfer arthritis), Tg197 (Transgenic mice that express stabilized TNFα mRNA on a C57BL/6 J strain), 11β-HSD1 (11 beta-hydroxysteroid dehydrogenase type 1), KO (Knock Out), TNF-tg11βKO (mice crosslinked for 11β-HSD1 and overexpression of TNFα mRNA), SJIA (systemic JIA), ON (Osteonecrosis), mS6KO (myeloid Sirtuin 6 KO), SF /Synovial Fluid), AP (Alkaline Phosphatase),

and TGF-β than the intimal lining layer, thus confirming the more mixed phenotypes observed in the synovial sublining layer.

Conversely, the cells $CD163^+FR\text{-}\beta^+$ in both layers also produce TNF-α [25].

Furthermore, from another study, similar observations were found. In fact, the cells $CD163^+$ M2 anti-inflammatory macrophages from synovial tissue when tested for protein production and expressed high levels of pro-inflammatory markers (PHD3, [CCR]-2, [MMP]-12 and TNF-α) [22].

Lastly, $CD14^+$ cells from RA synovial fluid express low levels of M2 anti-inflammatory markers, accordingly with a high level production of pro-inflammatory genes ([CCR]-2, [MMP]-12, and EGLN3 INHBA) [26,27]. Moreover, they possess high a pro-inflammatory polarization ability (induced by proteins such as activin A, PHD3 and [MMP]-12) [22].

3.1.3. Endogenous molecules that can modulate M1 and M2 polarization in rheumatoid arthritis

Macrophages, together with other specific cells (i.e. T-cells, fibroblasts), are responsible for joint swelling in RA, which reflects synovial tissue inflammation. While a balance between pro-inflammatory and anti-inflammatory factors is a condition for normal synovial tissue activities, in RA. On the contrary, an imbalance between producers and inhibitors of the inflammatory reaction is evident. In fact, a complex cytokine and chemokine network is involved in the inflammatory environment [28,29].

Among a total of eight articles it was found that different endogenous molecules were reported and highlighted as essential mediators of inflammation or, more importantly, crucial for maintaining the milieu homeostasis.

For example, the activity of CypA seems to induce pro-inflammatory signaling pathways in many specific cells, among which also macrophages. A growing body of evidence has suggested its pathological role in RA and many other human diseases [30].

CypA expression was detected in both RA and OA synovial tissue. The absolute concentration of CypA in RA was much higher than that in OA synovial tissues [31]. CypA induced the expression of cytokines/chemokines, such as TNF-α, IL-8, MCP-1, IL-1β and (MMP)-9, through a pathway that is eventually dependent on NF-kB activation [32]. The macrophage marker TSPO is a mitochondrial membrane protein which could be involved in a negative macrophage regulation during RA inflammation [33].

Moreover, MDM from synovial tissue showed a strong up-regulation of TSPO compared to their monocyte counterparts. Conversely, this expression was counterbalanced when synovial MDM were tested with M1 stimuli (LPS or IFN-γ). Interestingly, at both mRNA and protein level, TSPO showed a significant down-regulation by 24 h compared to those cells stimulated with IL-4, TGF-β or Dexamethasone [33].

Sirtuin 1 (SIRT)-1 is a histone/protein deacetylase which works in tandem with AMPK to control metabolism-transcriptional gene expression, neuroprotection, and inflammation. MDM from synovial tissue when activated with Resveratrol (RSV a (SIRT)-1 activator) can up-regulate the M2 transcripts (MDC, FcεRII, or CD23, MrC1, and IL-10) and suppress the M1 macrophages markers [(CCL)-2, iNOS, and IL-12 p40] [34].

The activity of (SIRT)-6 was found to be negatively correlated with IL-6 concentration in RA patients, and it was hypothesized that its enzymatic activity was strictly connected to the inflammatory process.

Furthermore, SEMA3A, a membrane protein with an axonal property, participates, among other activities, in bone homeostasis maintenance.

In fact, its concentration in RA synovial tissue seems significantly lower than that compared to the OA samples, and it is negatively correlated with RA disease progression in serum (DAS28 and RF [35,36]).

The expression of SEMA3A in vitro, derived from BMM and under polarization stimuli, seems to block the expression of the markers (iNOS)-2, ROS, the cytokines IL-1β, IL-6 and TNFα and boosted the increase of M2 mRNA specific markers expression Arg1, Fizz1, Ym1, CD206, CD163). Eventually, SEMA3A was found to promote the switch from M1 classically-activated macrophages to M2 alternatively-activated macrophages [37].

In fact, in synovial tissue, synovial fluid and PBMC-derived monocytes from RA patients rather than from OA patients, the (SIRT)-6 protein levels were significantly decreased [38].

Moreover, in order to define a specific μRNA expression profile in $CD14^+$ purified monocytes obtained from the peripheral blood of 27 JIA patients, an analysis of 110 μRNA was performed.

The results showed that miR-125a-5p was found to be highly up-regulated in vitro tests, and it drove the polarization of stimulated macrophages to anti-inflammatory (M2) counterparts. As a major result, it was shown that miR-125a-5p drove the polarization towards M2b and M2c sub-groups [39].

Comparable with this result, $CD14^+$ monocytes from 32 children with SJIA showed that the expression of miR-146a was increased and was correlated with systemic features. In addition, transfection experiments demonstrated that miR-146a is linked with the generation of alternatively-activated M2 macrophages (inducing the increase of the genes Arg1, (CCL)-17 and (CCL)-2 associated with the markers CD163 and CD206) [40].

Anti-citrullinated protein antibodies (ACPA) and auto-antibodies are very important in the diagnosis of RA because they can also predict disease severity [41]. The M1/M2 ratio imbalance seems to be associated with the number of OCs in $ACPAs^+$ RA Patients- Although ACPAs, ESR and CRP were found to be correlated with a M1/M2 ratio, the latter is the sole significant factor that contributes to the number of OCs [21].

Cells isolated from RA patients' synovial fluid are a high source of activin A, which promotes the production of GM-CSF-dependent polarization markers and inhibits the expression of M-CSF-dependent markers in both monocytes and macrophages, thus supporting its possible contribution to the prevalent pro-inflammatory polarization of macrophages (M1) in RA synovial tissues [22]. Synovial fluids from RA patients also contain two proteins involved in TLRs signaling, namely (IRF)-4 and (IRF)-5. ACPAs antibodies, purified from RA serum, seem to induce a significant increase in the M1/M2 ratio in vitro, as well as (IRF)-4 and (IRF)-5 at mRNA levels.

However, only (IRF)-5 from silencing experiments were found to be associated with the action of ACPAs [23]. Interestingly, the study by Kang et al. showed why IFN-γ drives the polarization of macrophages towards the M1 state [42].

Notwithstanding this mechanism being helped by numerous molecular processes [43,44], they found epigenomic evidence which shows how IFN-γ repressed several genes by disassembling the regulatory elements that control the gene expression (enhancers). Specifically, IFN-γ, from RA synovial macrophages, suppressed M2-related homeostatic and reparative genes binding the transcription factor called MAF, thus de-activating the upstream gene enhancer [42].

3.1.4. Exogenous molecules (drugs) that can modulate M1 and M2 polarization in rheumatoid arthritis

The migration of $CD68^+$ macrophages in the synovial tissue is a biomarker of both disease severity and results of therapeutic activities [45]. However, data relating to the consequences of RA bDMARDs on macrophage polarization are very limited. Anti-cytokine bDMARDs have been shown to decrease the inflammatory burden caused especially by recruited inflammatory monocytes/macrophages [46]. In fact, only one study analyzed the effect of bDMARDs modulation of M1 and M2 polarization [46].

Anti-TNF agents seem to provoke the modification in macrophage polarization from inflammatory to anti-inflammatory status, meaning the down-regulation of the surface markers M1-like, such as CD40 and CD80, to the up-regulation of the surface marker M2-like, such as CD16,

CD163 and MerTK. Additionally, anti-TNF agents prevent the production of inflammatory cytokines (TNF-α, IL-6, IL-12) and increase the phagocytosis of macrophages via the higher production of IL-10 which controls the inflammatory process by the activation of Gas6 and SOCS3.

Noteworthy, IL-10 mediates the switch from M1 to M2 through pSTAT3. The other bDMARDs that were studied did not induce any polarization into macrophage subsets [46].

Lastly, the stimulation of fresh monocytes with Pam3 [19], a (TLR)-2 agonist, did not significantly modify the classical surface marker profile of M2 macrophages. Conversely, LPS, a (TLR)-4 agonist, is responsible for the low-level activation of M2 markers against the high-level production of M1 markers (evaluated as MFI values). Likewise, the action of Pam3 down-regulated the mRNA M2 marker (FRβ)-2 and SLC40A1, but not HMOX1. The induction with LPS or LPS plus IFN-γ seems to amplify all mRNA markers mentioned above. Surprisingly, Pam3 induces a high production of pro-inflammatory cytokines (IL-1β, IL-6, and IL-8) in M2 macrophages. Stimulation of M1 and M2 with combination of Pam3 and LPS induces an up-regulation of (TLR)-2 and a down-regulation of (TLR)-4 [19].

3.2. Mice and rats

Due to the complexity of RA, there is still a lack of understanding of the fundamental causes of the disease. For example, the trigger to the beginning of the articular disease is still unknown, and the molecules that define chronicity versus resolution of inflammation are also poorly understood. Therefore, the use of animal models remains essential in order to delineate the molecular mechanisms of the disease so as to develop novel therapies.

To date, various mouse models are available which involve different immune cell types and which address distinct stages in the course of RA [47,48].

3.2.1. M1 and M2 from blood and synovial tissue of RA models

The K/B × N serum-transfer mouse model for STIA summarizes the onset phase of human RA, but it is independent on the adaptive immune response. The model depends on the immune-complex engagement of activating Fc receptors on macrophages, and IVIG preserve mice from the disease through the expression of inhibitory Fc receptors [49]. The work of Misharin et al. showed that non-classical $Ly6C^{-}$ monocytes (that correspond to human $CD14^{dim}CD16^{+}$ monocytes) are required for the induction and progression of sterile joint inflammation in the K/B × N mice RA model [50].

Non-classical $Ly6C^{-}$ monocytes polarize into inflammatory macrophages (M1), induce disease pathogenesis and demonstrate plasticity during the resolution phase [50].

Throughout the progression of arthritis, $Ly6C^{-}$ monocytes differentiate towards an alternatively activated phenotype (M2), promoting the joint inflammation resolution and all these steps occur without changes in the synovial tissue-resident macrophages [50].

In order to better characterize the gene expression of AIA in inflamed mice synovial tissue, a microarray analysis was carried out [51].

The M1 markers (IL-1β, IL-6, FcγRI and CD86) were strongly up-regulated during the course of AIA whereas the majority of M2 markers ([IL]-1RII, CD163, CD206, Arg1 and Ym1) were also somewhat up-regulated, with the exclusion of Arg1 and Ym1 which were exclusively and differentially expressed from the outset of AIA. In fact, this might once again indicate a change towards an M1 signature in the inflamed synovial tissue even during AIA [51].

Lastly, in the mouse model of SIO, a clinical condition simulating late RA, M1 macrophages increased during the beginning phase together with an altered M1/M2 ratio, while the expression of TNF-α remained high [52].

When the concentration of TNF-α decreased, and the tissue entered a resolution phase also the macrophages were found to have mainly shifted to the M2 anti-inflammatory state [52].

3.2.2. Endogenous molecules that can modulate M1 and M2 polarization in RA models

In total, six studies of both ex-vivo and in vitro studies were found in which different molecules drove PB and synovial monocytes/macrophages towards the polarization in M1 or M2 macrophages.

In the mouse model of (SIRT)-1-Tg CIA, a model very similar to human RA progression, Park et al. confirmed the data obtained from reported human experiments [34]. In this experiment, the (SIRT)-1 over-expressed mice were found to develop less severe arthritis than WT C57BL/6 N mice; noteworthy, the (SIRT)-1 over-produced augmented the release of M2 markers (i.e. arginase-1) and, decrease the concentration of M1 markers (i.e. TNF-α and IL-1β) [34].

The 11 beta-hydroxysteroid dehydrogenase type 1 (11β-HSD) enzyme transforms GCs inactive to their active counterparts, conferring tissue-specific increase and exposure to active endogenous and therapeutic GCs [53].

As matter of fact, a Tg197 mouse model was cross-linked with mouse line KO for 11β-HSD and it was found that they developed more severe arthritis than WT C57BL/6 J-induced arthritis mice and showed a larger infiltration of M1 macrophages in the synovial tissues. This data confirms the increase of pro-inflammatory cytokines (IL-1, IL-6, CSF1), and supports the anti-inflammatory properties of 11β-HSD [53].

Likewise, in a combination of in vitro cell cultures and a TNF-Tg arthritic mice model, carrying an Hes1-GFP transgene as an indicator of Notch activation, Sun et al. found that Notch signaling [54] has strength connection with M1 macrophage polarization and its inhibition reduces joint tissue inflammation by inducing a change from M1 to M2 macrophage [55].

Conversely, a mouse model depleted by IL-10 ($IL\text{-}10^{-/-}$) worsened CIA, and promoted macrophage polarization towards pro-inflammatory M1, confirming that IL-10 production could maintain the homeostasis between inflammatory and anti-inflammatory macrophages [56]. Moreover, a high amount of IL-10 provokes the switch from M1 to M2 macrophages [56].

In regards to the anti-inflammatory molecule, the study of Woo et al. confirmed the results obtained in RA patients. In fact, in a mouse model of (SIRT)-6 deficiency, CIA arthritis was induced and showed an expanded $CD68^{+}$ cell immunoreactivity in the intimal lining and sublining synovial tissue. Moreover, M1 macrophages linked genes, chemokines, and receptors and proteases were increased [38].

In line with human data, Zhu et al. found a mouse model, knocked out for (IRF)-5, produced a macrophage shift to its M2 anti-inflammatory counterparts [23]. In addition, they confirmed that $IRF5^{-/-}$ mice were lower producers of pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-12.

Furthermore, the intracellular staining of macrophages in the mouse model, knocked out for (IRF)-5, demonstrated that the transcription factor was more present in M1 than in M2 macrophages.

In addition, an ex-vivo study of AIA mice confirmed that the (IRF)-5 protein was higher in M1 pro-inflammatory than M2 ($CD206^{+}$) anti-inflammatory macrophages in the affected knee. Moreover, $CD206^{+}$ macrophages expressed more (IRF)-5 at the site of inflammation than they did before the challenge [57,58].

We can also consider data from Lyme-arthritis studies which are useful in extrapolating information about M1 and M2 macrophage behavior and indeed they might be comparable to RA studies. In fact, we found one study where Lyme-arthritis was induced in C3H/HeNCrl (CH3) and C57BL/6NCr (B6) mice infected by bacterial Borrelia burgdorferi–induced Lyme-arthritis [59]. In this study Ma et al. showed how Bbaa1 modulated the macrophage polarization [59].

The Bbaa1 B6 allele stimulated the expression of the M2 markers, whereas the Bbaa1 C3H allele promoted the typical production of M1 markers [59].

The studies eventually identified Bbaa1 as a regulator of type I IFN response during both Lyme-arthritis and RA development. This finding is consistent with the hypothesis that inherent patterns of gene

expression, controlled by Bbaa1 alleles, may possibly direct the functional phenotype of macrophages as classically (M1) ones versus alternatively (M2) polarized ones [59].

3.2.3. Exogenous molecules that can modulate M1 and M2 polarization in models of RA

It is possible that enhancements in the exploration of nanotherapeutic techniques might provide new solutions to bypass the defects of existing treatments. Therapeutic drugs can be selectively distributed to and accumulated in the inflamed sites via the passive or active targeting of nano-vehicles after systemic administration. This therapeutic method has, in fact, reduced the damage to other organs and the "vehicle" protects the drug until it reaches the burden site.

Eleven studies of therapeutic molecules that drive PB and synovial monocytes/macrophages towards polarization in M1 or M2 macrophages were herein analyzed.

3.2.3.1. Rats. In AIA rats, manganese ferrite and ceria nanoparticle-anchored mesoporous silica (MFC-MSNs) exhibit a synergistic effect on O_2 generation and ROS elimination, inducing a shift of M1 to M2 macrophage polarization both in vitro and in vivo [60].

In a rat model of induction of the AIA, a gene therapy, with IL-10 DNA plasmid encapsulated in nanoparticle carrying the protein tuftsin, was constructed to reach effectively synovial tissue macrophages.

The IL-10 gene therapy nanoparticle protein driven, induced a change of membrane polarization markers in synovial macrophage, from M1 to M2. In addition, the treatment induced systemic reduction of serum and synovial pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) [61].

Moreover, targeted delivery of Withaferin-A incorporated in mannosylated liposomes (ML-WA) ameliorated the gravity of inflammation in AIA rats, inducing the M1 to M2 macrophage polarization shift [62]. Instead Chang et al. have improved the bio-availability of monoterpene glucoside Pae by producing one novel ester: CP-25. The paeoniflorin-6′-O-benzene sulfonate (CP-25) have many beneficial activities in AIA rats. In fact, it repressed the production of pro-inflammatory cytokines (TNF-α, IL-1 and IL- 6), increasing the TGF-β1 concentration. The effects were similar to the effects of MTX alone. CP-25 treatment inhibited M1 macrophage production and increased M2 macrophage activation by inducing cytokine release [63].

Finally, alkaline phosphatase (AP) administration in arthritic rats, alone or in combination with MTX reduced the macrophages' infiltration level of $CD68^+$ and $CD163^+$ in the synovial tissue. The reduced number of infiltrated $CD68^+$ macrophage were statistically significant compared to normal rats [64].

3.2.3.2. Mice. In mouse model of CIA, sSiglec-9 significantly ameliorated the clinical and histological evidence and gravity of arthritis. While sSiglec-9 diminished the expression of M1 markers RelA/p65 in macrophages, it did not affect the expression of M2 markers [65].

Furthermore, the study of Li et al. [66], to obtain additional data in function of FUTs in RA polarization of macrophages, were supported with in vivo study in CIA mice. They found that there was a decrease in the percentages of $CD11b^+Ly6C^-$, $CD11b^+$TNF-α, and $CD11b^+$IL-23^+ M1 inflammatory macrophages in mice treated with fucose/galactose analog 2-D-gal. The higher amount of this molecule has been implicated in the shift M1 to M2 macrophage polarization state.

Instead, a micelle mixture, loaded with dexamethasone and siRNA against the NF-kB-p65 transcription factor, effectively inhibited NF-kB signaling in murine macrophages. The co-distribution method was efficient enough to switch macrophages from the M1 to the M2 state [67].

Furthermore, in order to better understand the role of CB2 on OC formation and differentiation in inflammation-induced cartilage and bone destruction, Zhu et al. tested JWH133, a selected agonist of CB2, in a CIA murine model. JWH133 treatment diminished the infiltration of pro-inflammatory M1-like macrophages in the knee joint and repolarized macrophages from the M1 to M2 phenotype [62]. The activation of CB2 increased the expression of anti-inflammatory cytokine interleukin IL-10 and reduced the expression of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6 [68].

Lastly, from an antagonist of CXCR3 (JN-2) was evaluated the efficacy on the modulation of (CXCL)-9/10/11, on the progression of arthritis in a CIA mouse model [69]. The results showed that the increased levels of TNF-α, IL-6, and RANKL (one of M1 Macrophages marker) were greatly inhibited. Additionally, the CIA-induced mRNA expression of TNFα, IL-6, and RANKL transcript (Tnfsf11) were blocked by JN-2-injected mice [69].

In the K/B × N serum transfer RA murine model (yielded from KRN strain crosslinked with NOD-SCID IL2R$\gamma^{-/-}$ strain) DMSO injection produced a contradictory result. In fact, the forepaws, but not the hind paws, expressed significantly lower levels of pro-inflammatory genes (IL-1β, IL-6, CXCL1 and CXCL2) after DMSO treatment. Surprisingly, in mice derived BMM DMSO acts to block the polarization of M0 macrophages into M1 or M2 phenotypes [70].

4. Discussion

In the present systematic review, detailed aspects of the activities and polarization properties of M1 and M2 macrophages have been reviewed for the first time in RA blood, synovial tissue and synovial fluids. The aim of the review is to give a comprehensive overview of the current literature available, presenting the results obtained from findings in humans, mice and rats, including endogenous or exogenous molecules which participate in inducing the polarization of macrophage (M1 or M2).

From the analysis of literature regarding RA models. it was revealed that in mice blood there is a non-classical $Ly6C^-$ monocyte population (human's $CD14^{dim}CD16^+$ monocytes) which are essential for the initiation and progression of arthritis at the level of inflammatory reactions. These cells, polarized in pro-inflammatory M1 macrophages, provoke tissue inflammation and differentiate into anti-inflammatory M2 macrophage cells to resolve the burning milieu [50].

Surprisingly, resident synovial macrophages do not change during inflammation; moreover, they do not present the MHCII marker ($MHCII^-CXCR3^+$), they populate the synovial tissue since the embryonic development, and they have a key role in maintaining its homeostasis [50].

On the other hand, Rossol et al. found that the intermediate $CD14^{2+}CD16^+$ human blood monocytes (corresponding to $Ly6C^{int}$ in mice) have a higher frequency in patients with RA than in HD, whereas non-classical $CD14^{dim}CD16^+$ monocytes (corresponding to $Ly6C^-$ in mice) are unchanged in RA [71].

Conversely, our findings in this systematic review analysis, using human phenotypical analyses, on three specific blood monocyte populations (i.e. classic $CD14^+CD16^-$, intermediate $CD14^{2+}CD16^+$ and non-classical $CD14^{dim}CD16^+$) reveal that there are no differences between RA and HD subjects [17].

Moreover, $CD14^+$ and $CD16^+$ populations did not change in JIA patients compared to HD subjects [18].

More detailed phenotypical and cytokine production analysis of PB cells also revealed that blood from RA and JIA patients is composed of a mixed M1 and M2 monocyte sub-population [17,18,20] while $Cd14^+$ monocytes show the M2 phenotype in the quiescence phase [21].

Although RA blood monocytes and activated macrophages do not show any differences compared to HD, our review reveals that there is a more complex situation in synovial tissues. Since mono IHC analysis reveals a subset marker disequilibrium (mixed M1/M2 markers) resulting from double immunofluorescence, we can confirm that the synovial tissue intimal lining layer shows more mature and defined macrophages, and it would seem that this tissue region has already started the resolution phase ($CD68^+$ colocalizing with $CD163^+$ and

$CD32^+$; $CD163^+$ macrophages colocalizing with FR-β [25], but producing much more IL-10 and TGF-β than TNF-α and iNOS) [17].

These findings were further endorsed by a ON mice study where scientists found a correlation between TNF-α decreasing and the onset of the resolution phase [52].

On the contrary, the sublining tissues show a much more mixed phenotype ($M\Phi_{IL\text{-}10}/M\Phi_{IFN\text{-}\gamma}$ mixed markers and cytokines) [17]. A gene expression study from rodents, during the course of AIA, showed that, although two M2 markers remained high and stable throughout the disease period (Arg1 and Ym1), M1 markers were strongly upregulated (IL-1β, IL-6, FcγRI and CD86) [51].

Eventually, comprehensive analyses of RA synovial fluid macrophages focused, in particular, on cell membrane receptors, genes and protein expressions, showed that in $CD14^+$ lineage, anti-inflammatory membrane markers were expressed at a low level while pro-inflammatory genes and proteins were produced at high levels [22]. However, contrary to this result, Palacios et al. also found that, from synovial tissue, M2 macrophage lineage expressing $CD163^+$ produces a high amount of proinflammatory proteins [22]. Moreover, the local inflammatory milieu in synovial fluid can block the expression of CD200R and CD163 markers by M2 macrophages [72]. (See Fig. 2 for a detailed illustration showing the distribution of macrophages in the layers of synovial tissue).

Based on these results, any possible limitations related to such an approach should be fully considered in order to understand the role of M1 and M2 polarization in RA.

However, since our intention with this review was to give the most complete overview possible of the literature on this somewhat difficult topic, only analyzed studies in which more than five RA patients were recruited have been included. However, the differences in the method of the cohort sample choice from different laboratories, for example, the definition of early/ active phase / remissive RA patients, or the characterization of the differences found between the therapies, has not allowed us to produce a meta-analysis of the data extracted from the studies selected.

Nevertheless, standardization advances have been made in the past, two examples being the studies from Bresnahan et al. and Smith et al. which both proposed the setting method in IHC evaluation and infiltration of macrophages from synovial tissue [73,74]. Furthermore, Murray et al. proposed guidelines and nomenclature for macrophage activation and polarization [75]. Therefore, it could now be helpful to have a development of standardized selection methods for collecting the patients' samples and find ways of how to choose the therapies for evaluating M1 and M2 in RA.

In animal models, there are various options that simulate human RA [76] but the problem with having several models is that it could create a limitation in producing results which are in contrast with each other. In fact, this would likely happen due to different mouse strains, genomes and different technical methods being used despite the attempts to develop conditions as similar as possible to the alteration caused by human RA.

For example, we found that K/B × N serum-transfer mice develop $Ly6C^-$ monocytes which determine the infiltration in synovial tissue, polarized as pro-inflammatory macrophages that can differentiate in anti-inflammatory macrophage to resolve the inflammatory burden [25].

However, in the AIA model, $Ly6C^+$ drives the inflammation [58], and, in this line, it is the $ly6C^+/(CCR)\text{-}2^+$ cells that control the autoimmune tissue alterations in the CIA model of RA [77]. These conflicting results in differing models of animal arthritis suggest that extrapolation to human RA is still premature and misleading.

Another related limitation is due to the fact that there is no one particular standardization method to choose from among the better considered techniques in order to evaluate macrophages taken from synovial tissue and blood. Furthermore, the precise definition of RA immuno-pathology (in particular, for the definition of macrophage activity) needs markers, protein, and gene standardization methods so as to produce reliable results. It is a known fact that laboratories around the world all too often use different panels of macrophage identification markers for both the blood and the synovial tissue [17,22,37].

In fact, comparing the different search methods, we could deduce how the classical division of M1 and M2 macrophages, by itself cannot help us to fully understand the analyzed population, and it should be supported by more precise techniques that truly evaluate a more accurate and in-depth the result [17].

To confirm this, an unexpected population with a membrane marker of anti-inflammatory M2 macrophages (i.e. $CD163^+$) contrarily expressed functional pro-inflammatory markers at mRNA and protein levels. Furthermore, the stimulation of macrophages with the TLR2 receptor antagonist did change the membrane markers, but the genes

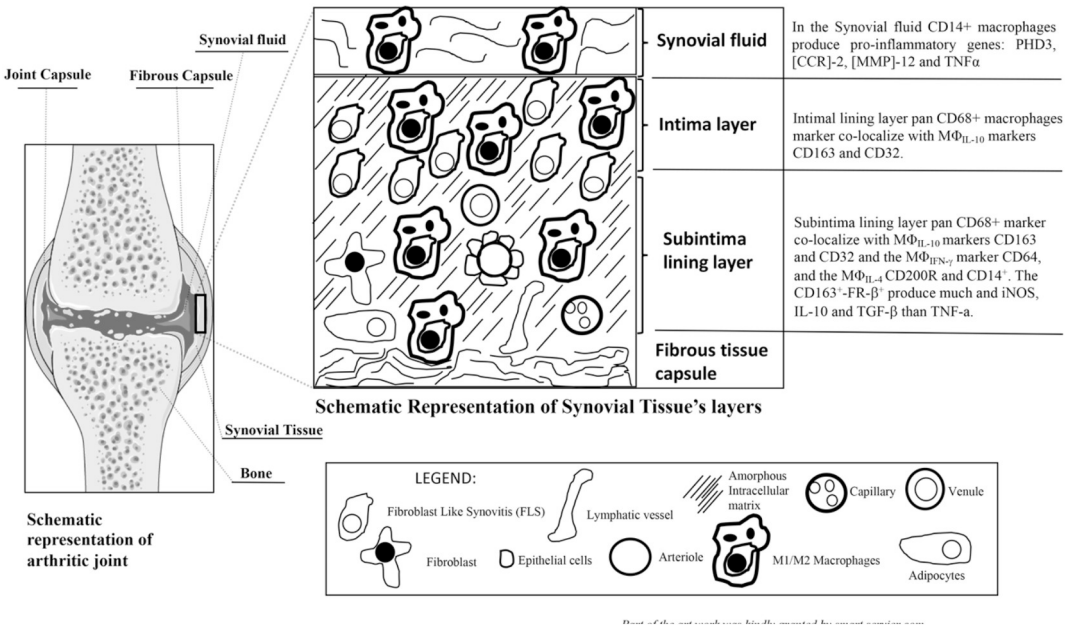

Fig. 2. Schematic representation of M1/M2 macrophages distribution in synovial tissue's layers [17,22].

and proteins constantly showed a pro-inflammatory profile [22,64].

Thus, there are still several unanswered questions:

Are membrane markers the best method to evaluate and define macrophage populations?

Is it still useful to use the classification based on markers for M1 and M2 macrophages? [78].

In relation to the tissue where the macrophages are extracted, does the function change?

In fact, the work of Ambarus et al. [17] described that, in synovial tissue, the cellular layer (intima) presented a more defined cluster of macrophage cells ($M\Phi_{IL\text{-}10}$ markers colocalized with $CD68^{+}$) compared to the vascular/fibrous synovial layer where cells seems more immature ($M\Phi_{IFN\text{-}\gamma}$/$M\Phi_{IL\text{-}4}$ & $M\Phi_{IL\text{-}10}$). Perhaps this is due to the proximity of blood vessels as this could create a higher level of cellular turnover. Taking this into account, we could suppose that the particular tissue environment (e.g. chemokines, cytokines produced by T cells, and FLS) possibly defines the plasticity of macrophages [79,80].

This hypothesis is supported by the proposal of Mosser et al., for which it would seem to be wiser to modify the nomenclature of the macrophages, changing the classical membrane marker nomenclature, and using a classification characterized on the fundamental macrophage function [78].

In this regard, the authors have proposed three functional classes for macrophages namely: the host defense, wound healing, and immune regulation. They explained that this classification method could be more helpful in illustrating how these cells can evolve, allowing scientists to more accurately identify the different macrophage levels of activation [78].

Thus, to better understand the milieu which induces the polarization towards the pro-inflammatory or the anti-inflammatory state, after the comprehensive overview of the literature, we have summarized the most important molecules that seem to drive the M1/M2 macrophage response in the RA disease.

Among anti-inflammatory molecules, TSPO seems negatively correlated with the progression of the disease [33]; (SIRT)-1, when activated can up-regulate the M2 macrophage transcript (human results compared with that of mice) [34] and (SIRT)-6 correlates negatively with systemic IL-6, and the in vivo (SIRT)-6 deficiency experiment confirms that it is negatively associated with the M1 response [38].

The action of (SIRT)-1 is similar to the action of ET-1 which was found to be involved in the expression of M2 markers (CD204, CD206, CD163, IL-10 and (CCL)-22) and in the production of TGFβ1 [81].

In addition, 11β-HSD promotes the activation of glucocorticoids (endogenous or exogenous), and shows anti-inflammatory properties in a knock-out experiment [53]. Confirmation of the same results were for IL-10 in Mice KO which had an aggressive production of M1 macrophages. Moreover, SEM3A, which has an axonal activity, can promote the switch from M1 to M2 macrophages [37].

In regards to regulating molecules, surprisingly two μRNAs are associated with the development of the anti-inflammatory response, namely, miR-125a-5p and miR-146a [39,40].

Conversely, Bae et al. found that both PB, synovial tissue and synovial fluid miR-146a levels are elevated in patients with RA, and that PB miR-146a levels correlate with clinical ESR values. They propose that miR-146a concentration plays a significant part in the proinflammatory process in RA [82]. Furthermore, Moran-Moguel et al. study proposed miRNA125a-5p as a potential biomarker to identify RA patients, and categorized miRNA146a, with its associated properties, with inflammatory macrophages in synovial tissue [83].

On the other hand, among the pro-inflammatory molecules, Activin-a [22] and two transcription factors in downstream activity of IFN-γ, namely, (IRF)-4 and (IRF)-5 [57]; (human and mouse) seem to be correlated with the development of the disease, with (IRF)-5 being more expressed in M1 than M2 macrophages [23].

In mouse cardiac (macrophage) nanotherapy, the use of the silencing siRNA for (IRF)-5 has also been tested, and it produced a relevant reduction of M1 macrophage as well as being seen to facilitate the resolution of the inflammatory reaction [57,84].

On the contrary, Kang et al. showed new and complex ways on how IFN-γ may block the expression of M2 genes and favor the pro-inflammatory expression ones [42].

Furthermore, among the exogenous molecules/drugs approved for RA treatment, bDMARDs were tested for the modulation of macrophage polarization; only ETA, and ADA, both anti-TNF-α agents, have induced the polarization of macrophage towards alternative anti-inflammatory features by modulating the polarization of macrophages, both on the activation of surface markers and on the expression of mRNA and cytokines characterizing M2 [46].

In addition, among the bDMARDs, of particular interest is the fusion protein CTLA-4-Ig (abatacept) which blocks the interaction between CD80/86 and CD28 on T cells and mediates immunosuppressive activities. However, data generated from studies about this drug and macrophages' polarization to date is very little.

CTLA-4-Ig modulates the production of pro-inflammatory cytokines (such as IL-6, IL-1β, TNF-α), both extracellular and cytoplasmic; also by down-regulating the same pro-inflammatory transcript (mRNA) [85–87]. In addition, abatacept can exert its function on different cells inside the inflammatory milieu [88,89]. Nevertheless, reliable data on abatacept activity to induce a shift from M1 pro-inflammatory to M2 anti-inflammatory macrophages needs to be further investigated.

Apart from bDMARDs, we found different fused macromolecules (nanoparticle or micelles mixtures) or novel specific antagonists (all tested in animal models) which can block the pro-inflammatory M1 or can polarize the macrophages to the anti-inflammatory M2 response [51,60–66,68–70,90–92].

Therefore, the future direction of nanomedicines in RA and continued research of new potential molecules might well improve the negative patients' response, by reducing toxicity and increasing therapeutic efficacy [93].

5. Conclusions

Present systematic review indicates that in blood and, more specifically, in the synovial tissue of RA patients (and animal models), there is a macrophage disequilibrium with M1 and M2 markers both present on the surfaces of the cells.

In fact, the specific surface-cell markers seem to not always be coherent with the functional status of these cells (with pro or anti-inflammatory activity). Moreover, markers at a mRNA and protein (cytokine) level show a phenotype dissociated from what should be expected from surface-cell markers (M1 or M2).

In regards to this point, it is most important to highlight that the role of the endogenous environmental factors, related to the circadian rhythms of the immune-inflammatory reaction, and the related hormonal and cytokine changes, deeply influence cell distribution and reactivity in the body [94–98].

Noteworthy, the production of inflammatory cytokines by resident and activated synovial cells, could strongly modulate the plasticity of macrophages to assume the most recent polarizing condition independent of their previous polarization state [79,80].

To conclude, we can suggest that more detailed investigations on M1 and M2 macrophage functions are needed, as well as on their modulation in RA, as this is essential not only for a better understanding of the patho-physiological and clinical features of the disease, but also to ensure a better therapeutic management of patients [88].

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ST and GM did substantial contributions to the design of the study, the acquisition, analysis and interpretation of data, the drafting of the article, and the final approval of the version to be published.

SS worked on the interpretation of the data and provided an important scientific point of view in the critical revising of the article.

SP, GP, MP, EA and VM provided an important clinical point of view in the critical revising of the article before its final approval.

MC was responsible for the conception of the study, revising the critically article for its important intellectual content and final approval of the version to be submitted.

Declaration of Competing Interest

All authors state that they have no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations.

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Appendix A. Supplementary data

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