Review

Cells of the synovium in rheumatoid arthritis
Macrophages

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Abstract

The multitude and abundance of macrophage-derived mediators in rheumatoid arthritis and their paracrine/autocrine effects identify macrophages as local and systemic amplifiers of disease. Although uncovering the etiology of rheumatoid arthritis remains the ultimate means to silence the pathogenetic process, efforts in understanding how activated macrophages influence disease have led to optimization strategies to selectively target macrophages by agents tailored to specific features of macrophage activation. This approach has two advantages: (a) striking the cell population that mediates/amplifies most of the irreversible tissue destruction and (b) sparing other cells that have no (or only marginal) effects on joint damage.

Introduction

Macrophages (Mϕ) are of central importance in rheumatoid arthritis (RA) due to their prominent numbers in the inflamed synovial membrane and at the cartilage-pannus junction, their clear activation status [1,2] (see Table 1 for overview), and their response to successful anti-rheumatic treatment [3]. Although Mϕ probably do not occupy a causal pathogenetic position in RA (except for their potential antigen-presenting capacity), they possess broad pro-inflammatory, destructive, and remodelling potential and contribute considerably to inflammation and joint destruction in acute and chronic RA. Also, activation of this lineage extends to circulating monocytes and other cells of the mononuclear phagocyte system (MPS), including bone marrow precursors of the myelomonocytic lineage and osteoclasts [2,4,5].

Thus, before a causal factor for RA is known, monocytes/Mϕ remain an attractive research focus for the following reasons: (a) the radiological progression of joint destruction correlates with the degree of synovial Mϕ infiltration [1], (b) the therapeutic efficacy of conventional anti-rheumatic therapy coincides with downregulation of MPS functions [6], (c) therapies directed at cytokines made predominantly by Mϕ are effective in RA [7], (d) conventional or experimental drugs can be selectively targeted to Mϕ or their different subcellular compartments (for example, [2,8]), (e) differential activation of intracellular signal transduction pathways underlies different Mϕ effector functions [9], and (f) more specific inhibitors of key metabolic enzymes or particular signal transduction pathways may become available as selective targets of anti-rheumatic therapy [9,10]. In addition, the amplifying role of Mϕ in RA has emerged so clearly that the effects of anti-rheumatic therapy (whether specific or conventional) on monocytes/Mϕ may become an objective readout of the effectiveness of treatment [11-13] (Stuhlmuller B, Hernandez MM, Haeupl T, Kuban RJ, Gruetzkau A, Voss JW, Salfeld J, Kinne RW, Burmester GR, unpublished data).

Differentiation and activation of the mononuclear phagocyte system in rheumatoid arthritis

Cells of the myelomonocytic lineage differentiate into several cell types critically involved in disease (that is, monocytes/Mϕ, osteoclasts, and dendritic cells) (Figure 1a). Due to their marked plasticity, these pathways can be influenced by an excess/imbalance of cytokines or growth factors, resulting in altered differentiation/maturation (Figure 1b). In RA, such imbalances clearly occur in inflamed joints, peripheral blood, and bone marrow (Table 2 and Figure 1b).

AP-1 = activator protein-1; CRP = C-reactive protein; GM-CSF = granulocyte macrophage colony-stimulating factor; IFN = interferon; IL = interleukin; IL-1RA = interleukin-1 receptor antagonist; LPS = lipopolysaccharide; Mϕ = macrophage(s); MIF = migration inhibitory factor; MMP = metalloprotease; MPS = mononuclear phagocyte system; NF = nuclear factor; PPR = pattern-recognition receptor; RA = rheumatoid arthritis; ROS = reactive oxygen species; SEB = staphylococcal enterotoxin B; TGF-β = transforming growth factor-beta; TIMP = tissue inhibitor of metalloprotease; TLR = Toll-like receptor; TNF = tumor necrosis factor; TNF-R1 = tumor necrosis factor receptor 1; TNF-R2 = tumor necrosis factor receptor 2.

Table 1

Activation status of synovial macrophages and/or circulating monocytes in rheumatoid arthritis

Class of overexpressed molecules	Molecules	Known or potential function
Class II major histocompatibility complex (overexpressed on Mϕ)	HLA-DR	Presentation of antigens relevant to disease initiation or severity [93] (Stuhlmuller B, *et al*., unpublished data) (reviewed in [2])
Cytokines and growth factors	For example, TNF-α, IL-1, IL-6, IL-10, IL-13, IL-15, IL-18, migration inhibitory factor, granulocyte macrophage colony-stimulating factor, and thrombospondin-1	Mediation and regulation of local and systemic inflammation and tissue remodelling (reviewed in [2,24,39,52])
Chemokines and chemoattractants	For example, IL-8, macrophage inflammatory protein-1, monocyte chemoattractant protein-1, and CXCL13	Mediation and regulation of monocyte migration Stimulation of angiogenesis (reviewed in [69])
Metalloproteases (MMPs)	MMP-9 and MMP-12	Tissue degradation and post-injury tissue remodelling [94,95]
Tissue inhibitors of MMP (TIMPs)	TIMP-1	Attempt to control excessive tissue destruction [96]
Acute-phase reactants	For example, C-reactive protein and A-SAA (serum amyloid A)	Integrated hormone-like activation of hepatocytes by synovial Mϕ and fibroblasts (mostly via IL-6) [97] (reviewed in [2])
Other molecules	Neopterin	Produced by interferon-gamma-stimulated monocytes/Mϕ Induces/enhances cytotoxicity and apoptosis Acts as antioxidant [98,99]
	Cryopyrin	Produced by TNF-α-stimulated Mϕ Regulates nuclear factor-kappa-B and caspase-1 activation [100]

IL, interleukin; Mϕ, macrophages; TNF-α, tumor necrosis factor-alpha. Reproduced with permission from Kinne RW, Stuhlmuller B, Palombo-Kinne E, Burmester GR: The role of macrophages in rheumatoid arthritis. In *Rheumatoid Arthritis*. Edited by Firestein GS, Panayi GS, Wollheim FA. New York: Oxford University Press; 2006:55-75 [2].

Cells of the MPS show clear signs of activation, not only in synovial and juxta-articular compartments such as the synovial membrane or the cartilage-pannus and bone-pannus junctions (including the subchondral bone), but also in extra-articular compartments (for example, peripheral blood and subendothelial space, the latter of which is the site of foam cell formation and development of atherosclerotic plaques in RA) (Table 2). This activation underlines the systemic inflammatory character of RA and may contribute to the occurrence of cardiovascular events and its increased mortality (reviewed in [2,14,15]).

Biological functions of monocytes/macrophages and their role in rheumatoid arthritis

The monocyte/Mϕ system represents an integral part of the natural immune system and participates in the first-line response against infectious agents. Another crucial contribution to the body's homeostasis is the scavenging function of any debris generated by physiological or pathological processes. Thus, monocytes/Mϕ possess multiple and powerful biological functions that may greatly affect onset and development of chronic inflammatory diseases like RA (see overview in Table 3) (reviewed in [16]).

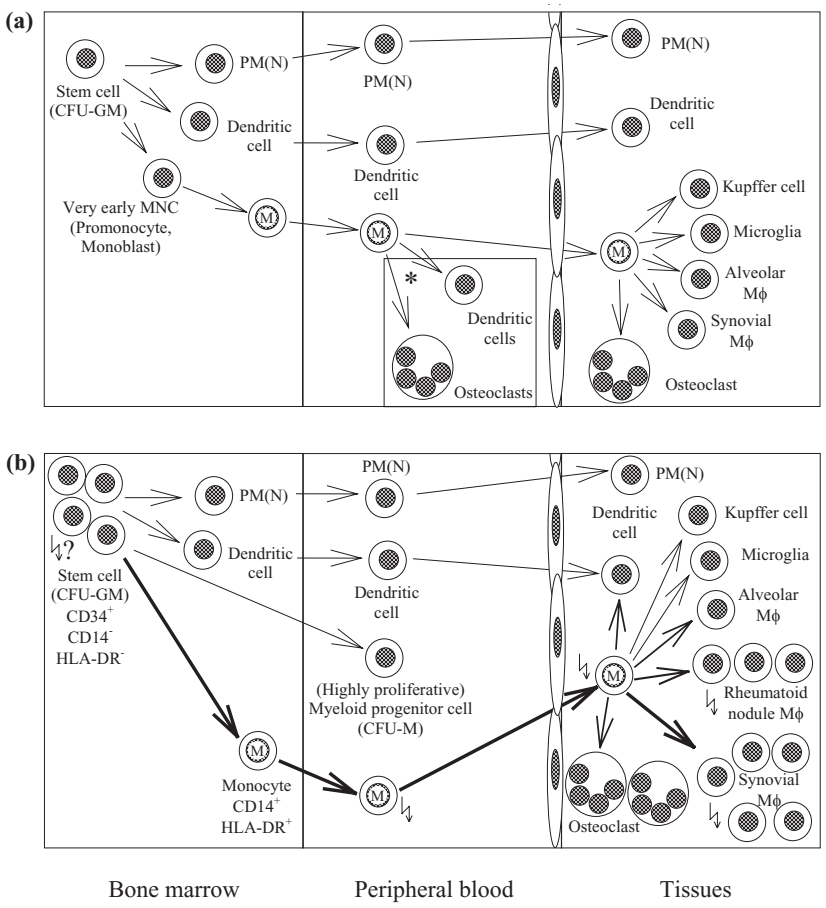
Stimulation/regulation of monocyte/macrophage activation in rheumatoid arthritis

The role of monocytes/Mϕ in RA is conceivably the integrated result of stimulatory, effector, dually active, and autoregulatory mediators/mechanisms. At the tissue level, the scenario is characterized by the influx of pre-activated monocytes, their maturation into resident Mϕ, their full activation, and their interaction with other synovial cells. The complexity of the interaction is the result of paracrine activation mechanisms generated via sheer cell-cell contact as well as of numerous autocrine mechanisms - nearly any soluble mediator shows abnormalities. A simplified scheme of this integrated system and the currently known mediators is provided in Figure 2. For ease of presentation, the parts are organized as incoming stimuli (both paracrine and soluble) (column a) and effector molecules (column b), although autocrine loops are also relevant (as discussed below).

Cell-cell interaction

A significant part of Mϕ effector responses is mediated by cell contact-dependent signalling with different inflammatory or mesenchymal cells (as exemplified in the lower left quadrant of Figure 2).

Figure 1



Physiological/pathological differentiation of the mononuclear phagocyte system in rheumatoid arthritis (RA). **(a)** Physiological differentiation of the mononuclear phagocyte system (MPS) (steady-state cytokine and growth factor milieu). In the human MPS, monocytes (M) differentiate from a $CD34^+$ stem cell via an intermediate step of monoblasts. Monocytes leave the bone marrow and remain in circulation for approximately 3 days. Upon entering various tissues, they differentiate into different types of resident macrophages (Mϕ), including synovial macrophages. It is believed that these mature cells do not recirculate, surviving for several months in their respective tissues until they senesce and die. Some circulating monocytes retain the potential for differentiating into dendritic cells and osteoclasts (asterisk in the insert). The steady-state myeloid differentiation involves many factors, including granulocyte macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-1, IL-6, and tumor necrosis factor-alpha (TNF-α), which are produced by resident bone marrow macrophages (reviewed in [2]). **(b)** Increased plasticity of myeloid differentiation and its possible role in RA (augmented cytokine and growth factor milieu). Human bone marrow intermediate cells can differentiate into macrophages or dendritic cells in the presence of c-kit ligand, GM-CSF, and TNF-α. TNF-α, in turn, inhibits the differentiation of monocytes into macrophages *in vitro* and, together with GM-CSF, directs the differentiation of precursor cells into dendritic cells, another important arm of the accessory cell system. Also, either IL-11 or vitamin D_3 and dexamethasone induce the differentiation of bone marrow cells or mature macrophages into osteoclasts, cells involved in the destruction of subchondral bone in RA. Osteoclasts and dendritic cells can also be derived from circulating monocytes upon stimulation with macrophage colony-stimulating factor (M-CSF) or IL-4 plus GM-CSF. This plasticity, and its dependence on growth factors or cytokines that are clearly elevated in peripheral blood and bone marrow of patients with RA, may explain some differentiation anomalies in the disease and also the efficacy of some anti-rheumatic drugs. Non-specific enhancement of monocyte maturation and tissue egression, in turn, are consistent with the known alterations in inflammation (reviewed in [2]). The differentiation paths potentially relevant to RA are indicated by bold arrows. The jagged arrows represent possible sites of cell activation. CFU-GM, colony-forming units-granulocyte macrophage; CFU-M, colony-forming units-macrophage; MNC, mononuclear cells; PM(N), polymorphonuclear leukocytes. Reproduced with permission from Kinne RW, Stuhlmuller B, Palombo-Kinne E, Burmester GR: The role of macrophages in rheumatoid arthritis. In *Rheumatoid Arthritis*. Edited by Firestein GS, Panayi GS, Wollheim FA. New York: Oxford University Press; 2006:55-75 [2].

Fibroblast-macrophage interaction

Because of the prominent numbers of Mϕ and fibroblasts and their activated status in RA synovial tissue, the interaction of these cells is critical for the resulting inflammation and tissue damage. Indeed, the mere contact of these cells elicits the production of interleukin (IL)-6, granulocyte macrophage colony-stimulating factor (GM-CSF), and IL-8. The cytokine output can be enhanced or down-modulated not only by addition of pro-inflammatory or regulatory cytokines (for example, IL-4, IL-10, IL-13, or IL-1 receptor antagonist [IL-1RA]),

Table 2

Potential sites of myelomonocytic activation in rheumatoid arthritis and corresponding steps of macrophage intermediate or terminal (trans)differentiation

Compartment	Location	Differentiation step
Joint or juxta-articular	Synovial membrane	• Recently immigrated monocytes • Mϕ (M1/M2? [64]; resident/inflammatory? [13]) • Dendritic cells
	Cartilage-pannus junction	Mϕ
	Subchondral bone	Osteoclasts
	Vascular endothelium	-
Extra-articular	Peripheral blood	Circulating monocytes
	Bone marrow	• Myelomonocytic precursors • Endothelial cells
	Subendothelial space	Mϕ / foam cells / pericytes
	Rheumatoid nodules	Epitheloid cells and multinucleated giant cells
	Lung interstitial space	Alveolar Mϕ

Mϕ, macrophages. Reproduced with permission from Kinne RW, Stuhlmuller B, Palombo-Kinne E, Burmester GR: The role of macrophages in rheumatoid arthritis. In *Rheumatoid Arthritis*. Edited by Firestein GS, Panayi GS, Wollheim FA. New York: Oxford University Press; 2006:55-75 [2].

but also by neutralization of the CD14 molecule [17]. Also, *in vitro*, significant cartilage degradation occurs in co-cultures of mouse fibroblasts and Mϕ, a response markedly exceeding that observed with each culture alone (reviewed in [2]). Furthermore, purified human synovial fibroblasts co-cultured with myelomonocytic cells induce cartilage degradation *in vitro*, but with a strong contribution of soluble IL-1 and tumor necrosis factor (TNF)-α [18].

T cell-macrophage interaction

Accessory, inflammatory, effector, and inhibitory Mϕ functions can be stimulated by fixed T cells or their plasma membranes if T cells are pre-activated and express activation surface molecules. In response to such interaction, monocytes produce metalloprotease (MMP), IL-1α, and IL-1β [19,20]. Also, T cells pre-stimulated in an antigen-mimicking fashion stimulate TNF-α and IL-10 production once in contact with monocytes [20]. Conversely, fixed T cells stimulated in an antigen-independent fashion (that is, with IL-15, IL-2, or a combination of IL-6 and TNF-α, the so-called Tck cells) induce monocyte production of TNF-α but not the anti-inflammatory IL-10 [20,21]. These findings suggest that early RA may reflect antigen-specific T cell-Mϕ interactions [22]. Conversely, chronic RA may be associated with antigen-independent interactions dominated by an exuberant cytokine milieu and Tck cells. This may also explain the relative paucity of IL-10 in the synovial membrane in chronic RA, as discussed below.

Several ligand pairs on T cells and monocytes/Mϕ have been implicated in this interaction [20], although the importance of individual ligand pairs, as well as the influence of soluble mediators, remains unclear. Interestingly, T cells isolated from RA synovial tissue show phenotypical and functional features similar to Tck cells and the above-mentioned signal transduction pathways differentially contribute to the induction of TNF-α and IL-10 production in monocytes/Mϕ by co-culture with Tck cells. If applicable *in vivo* in RA, this would allow selective therapeutic targeting of pro-inflammatory TNF-α and sparing of anti-inflammatory IL-10.

Interaction of macrophages with endothelial cells and natural killer cells

The interaction between monocytes and endothelial cells in RA (Figure 2), critical for the sustained influx of activated monocytes in the synovial membrane, relies on the altered expression of integrin/selectin pairs on the surface of the two cell types (reviewed in [2]). Because the synovial cytokine milieu (including the Mϕ-derived TNF-α) upregulates the expression of these ligand pairs, a self-perpetuating cycle ensues by which sustained Mϕ-derived mechanisms lead to further influx and activation of circulating monocytes. Upon cell contact, monokine-activated $CD56^{bright}$ natural killer cells induce monocytes to the production of TNF-α, thus representing another possible reciprocal loop of activation in RA [23].

Soluble stimuli

Cytokine stimuli with pro-inflammatory effects on macrophages

Numerous cytokines with known or potential stimulatory activity on monocytes/Mϕ have been identified, as schematically shown in the upper left quadrant of Figure 2. A systematic list of these stimuli and their known or potential functions is provided in Table 4. Some of these mediators are produced by monocytes/Mϕ themselves and therefore activate Mϕ in an autocrine fashion, as also exemplified in

Table 3

Monocyte/macrophage functions and their (potential) role in rheumatoid arthritis

Function	Mechanisms	(Potential) role in rheumatoid arthritis
Clearance of immune complexes	Binding of immunoglobulins to Fc receptors (Fc-γ-R I, IIA, IIB, and IIIA)	Potential clearance of rheumatoid factor but further activation of monocytes/Mϕ Opsonization of complexes by complement, leading to binding to Mϕ complement receptors and further cell activation [101,102] (reviewed in [2,103]) Notably, inhibition of monocyte activation by Fc-γ-R IIB [102]
Complement activation	Binding of complement factors to complement receptors 1 (CD35), 3 (CD11b), and 5a (CD88)	Recognition of activated complement (soluble phase or on immunoglobulin G-immune complexes) Promotion of phagocytosis and activation of monocytes/Mϕ [103]
Phagocytosis of particulate antigens	Conventional (Fc-mediated) → lysosomal degradation and MHC-II antigen processing	Scavenging of debris but potential import of arthritogenic molecules [103] Antigen presentation and activation of $CD4^+$ and $CD8^+$ T cells, possibly relevant to disease initiation or perpetuation (spreading of autoimmunity) (reviewed in [2])
	Coiling phagocytosis → lysosomal degradation and MHC-I antigen processing	Involved in phagocytosis of *Borrelia burgdorferi*, active agent of Lyme arthritis (reviewed in [2])
Clearance of intracellular pathogens and apoptotic cells	Removal of pathogens and recognition of apoptotic cells via exposed intracellular membrane components	Induction of Mϕ-derived cytokines by bacterial toxins or superantigens [26,28,103] Modulation of Mϕ responses by mycobacterial lipoarabinomannan [104,105] or Toll-like receptors [29,106] Persistence of obligate/facultative intracellular pathogens with arthritogenic potential [107,108]
Antigen processing and presentation	Enzymatic degradation of antigens and binding of antigenic peptides to MHC molecules and transport to the cell surface	Important cognate functions upon antigen recognition via presentation of antigen on MHC-II molecules [109] and expression of membrane second signal molecules adjacent to T cells (reviewed in [2])
Chemotaxis and angiogenesis	Attraction of other inflammatory cells and induction of neo-vascularization	Positive feedback between Mϕ-derived cytokines and chemotactic factors (for example, IL-8 and monocyte chemoattractant protein-1) Promotion of angiogenesis by IL-8 and soluble forms of adhesion molecules (for example, vascular cell adhesion molecule-1 and endothelial-leukocyte adhesion molecule-1) [69]
Wound healing	Remodelling of tissue via interaction with fibroblasts	Sustained monocyte recruitment at wound injury sites via monocyte chemoattractant macrophage inflammatory protein-1α Phagocytosis of matrix debris and endogenous production of IL-1, TNF-α, and so on as well as post-injury tissue remodelling (reviewed in [2])
Lipid metabolism	Mϕ synthesis of prostaglandins (PGs) E_2 and I_2 Expression of scavenger receptor A (uptake of oxidized low-density lipoprotein)	Pro-inflammatory activity of PGE_2 and PGI_2 and leukotrienes in rheumatoid arthritis, but also autocrine negative feedback through peroxisome proliferator-activated receptors α and γ (reviewed in [2]) Fish-based diets are associated with clinical improvement of human and experimental arthritis (reviewed in [2]) Modulation of T cell-contact-induced production of IL-1β and TNF-α in Mϕ by apolipoprotein A-I [110]

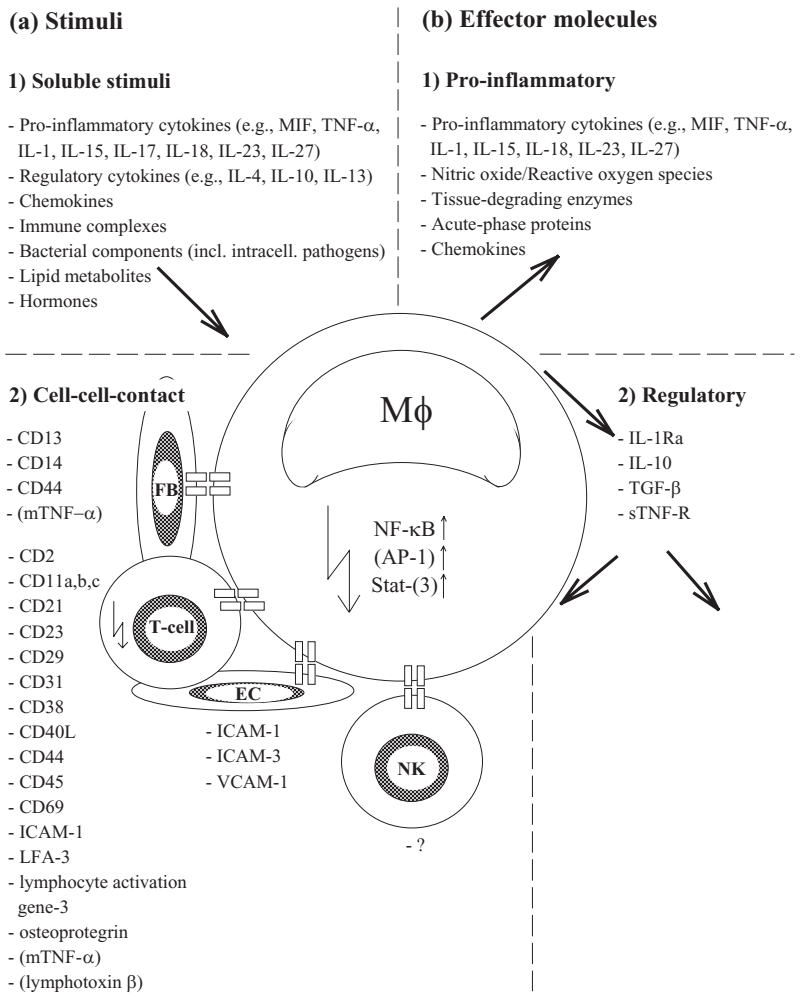
IL, interleukin; Mϕ, macrophage(s); MHC, major histocompatibility complex; TNF-α, tumor necrosis factor-alpha. Reproduced with permission from Kinne RW, Stuhlmuller B, Palombo-Kinne E, Burmester GR: The role of macrophages in rheumatoid arthritis. In *Rheumatoid Arthritis*. Edited by Firestein GS, Panayi GS, Wollheim FA. New York: Oxford University Press; 2006:55-75 [2].

Table 4. T-cell cytokines acting on Mϕ (for example, IL-17) have been comprehensively reviewed elsewhere [24,25].

Bacterial/viral components and Toll-like receptors

The ability of bacterial toxins or superantigens to initiate the secretion of Mϕ-derived cytokines is relevant in view of a possible microorganism etiology of RA and in view of side effects of anti-TNF-α therapy, particularly mycobacterial infections [26,27]. Lipopolysaccharide (LPS), for example, binds to Mϕ through the CD14/LPS-binding protein receptor complex and, *in vitro*, stimulates the production of IL-1β, TNF-α, and macrophage inflammatory protein-1α. Staphylococcal enterotoxin B (SEB), a potent Mϕ activator, enhances arthritis in MRL-lpr/lpr mice. Anti-TNF-α therapy, in this case, reverses both the severe wasting effects of SEB and the incidence of arthritis, indicating that TNF-α is central in this system. Finally, the staphylococcal enterotoxin A increases the expression of the Toll-like receptor (TLR)-4 in human

Figure 2



Paracrine, juxtacrine, and autocrine stimuli (column a) and effector molecules (column b) of macrophage (Mϕ) activation in rheumatoid arthritis. Most of the regulatory products of activated macrophages act on macrophages themselves, creating autocrine regulatory loops whose dysregulation possibly promotes disease severity and chronicity. The jagged arrow in the T cell indicates the necessity of pre-activating T cells for effective juxtacrine stimulation of macrophages. AP-1, activation protein; EC, endothelial cells; FB, fibroblasts; ICAM, intracellular adhesion molecule; IL, interleukin; IL-1RA, interleukin-1 receptor antagonist; LFA-3, lymphocyte function-associated antigen-3; MIF, migration inhibitory factor; mTNF-α, mouse tumor necrosis factor-alpha; NF-κB, nuclear factor-kappa-B; NK, natural killer cells; sTNF-R, soluble tumor necrosis factor receptor; TGF-β, transforming growth factor-beta; TNF-α, tumor necrosis factor-alpha; VCAM-1, vascular cell adhesion molecule-1. Reproduced with permission from Kinne RW, Stuhlmuller B, Palombo-Kinne E, Burmester GR: The role of macrophages in rheumatoid arthritis. In *Rheumatoid Arthritis*. Edited by Firestein GS, Panayi GS, Wollheim FA. New York: Oxford University Press; 2006:55-75 [2].

monocytes by ligation of major histocompatibility complex-II, with subsequent enhancement of pro-inflammatory cytokines by known TLR-4 ligands (for example, LPS [28]).

TLRs are part of the recently discovered cellular pattern-recognition receptors (PPRs) involved in first-line defense of the innate immune system against microbial infections. In addition to bacterial or viral components, some PPRs recognize host-derived molecules, such as the glycoprotein gp96, nucleic acids, hyaluronic acid oligosaccharides, heparan sulfate, fibronectin fragments, and surfactant protein A (reviewed in [29]). In RA, notably, functional TLR-2 and TLR-4 are expressed on $CD16^+$ synovial Mϕ, peripheral blood mononuclear cells, and synovial fibroblasts [30]. Also, their expression can be upregulated by cytokines present in the inflamed RA joint (for example, IL-1β, TNF-α, macrophage colony-stimulating factor, and IL-10); this suggests that activation of synovial cells via TLRs may contribute to disease processes [29], as supported by findings in experimental arthritis [31]. On the other hand, the chronic polyarthritis observed in mice with deletion of the DNase II gene, whose Mϕ are incapable of degrading mammalian DNA, appears to occur independently of the nucleic acid-specific TLR-9 [32].

Table 4

Overview of pro-inflammatory interleukins relevant to macrophage (dys)function in rheumatoid arthritis

Family	Cytokine	Pro-inflammatory	Dual	Autocrine	Main pathogenetic features
IL-1	IL-1	X	-	X	Predominantly produced by Mϕ Critical mediator of tissue damage Possesses autocrine features [43,51-53]
	IL-18	X	-	X	Predominantly produced by Mϕ Critical pleiotropic mediator of disease Possesses autocrine features [59-61]
	IL-33	X	X	-	Produced by endothelial cells Important Th_2-inducing component in allergy/autoimmunity Signals via IL-1 receptor-related protein (ST2) Nuclear factor with transcriptional repressor properties (≈ nuclear factor from high endothelial venules) [111-113]
IL-18 inducible	IL-32	X	-	-	Pro-inflammatory effects on both myeloid and non-myeloid cells [114,115]
IL-2	IL-7	X	-	-	Elevated in RA, although a relative paucity is also possible [116,117] Induces osteoclastic bone loss in mice [118]
	IL-15	X	-	X	Produced by Mϕ Important autocrine mediator of disease processes [21,56-58]
	IL-21	X	-	-	Only IL-21R is expressed by synovial Mϕ and fibroblasts [119]
IL-6	IL-6	X	X	-	Predominantly produced by fibroblasts under the influence of Mϕ Most strikingly elevated cytokine in acute RA, with phase-dependent differential effects [17,75,76] (reviewed in [2,77])
	IL-31	X	-	-	Induces experimental dermatitis [120]
	LIF	X	-	-	Stimulates proteoglycan resorption in cartilage [121]
	Oncostatin M	X	-	-	Recruits leukocytes to inflammatory sites and stimulates production of metalloprotease (MMP) and tissue inhibitor of MMP [121]
IFN type I/ IL-10	IL-19	X	-	X	Involved in both Th_1 and Th_2 inflammatory disorders [122,123] Possesses autocrine features [124,125]
	IL-20	X	-	X	Overexpressed in psoriasis Possesses autocrine features [122]
	IL-22	X	-	-	Relevant to innate immunity and acute-phase response [126]
	IL-24	X	-	-	Possible antagonism with regulatory IL-10 [127]
	IL-26	X	-	-	Polymorphism possibly contributes to RA sex-bias susceptibility [128]
	IL-28, IL-29	X	-	X	Involved in microbial recognition by upregulation of Toll-like receptors Possesses autocrine features [30,129,130]
IL-12	IL-12	X	-	-	Predominantly produced by synovial Mϕ and dendritic cells Promotes Th_1 responses (reviewed in [62])
	IL-23	X	-	-	Predominantly produced by synovial Mϕ and dendritic cells Shares p40 subunit with IL-12 and possibly antagonizes IL-12 [63] (reviewed in [62])
	IL-27	X	X	X	Produced by Mϕ and its neutralization has anti-arthritic effects Possesses autocrine features [66] Pro-inflammatory role [67]
IL-17	IL-17	X	-	-	Th_0-Th_1 lymphokine with pleiotropic, amplifying effects on Mϕ in arthritis (reviewed in [24,25])

IFN, interferon; LIF, leukemia inhibitory factor; Mϕ, macrophages; RA, rheumatoid arthritis. Reproduced with permission from Kinne RW, Stuhlmuller B, Palombo-Kinne E, Burmester GR: The role of macrophages in rheumatoid arthritis. In *Rheumatoid Arthritis*. Edited by Firestein GS, Panayi GS, Wollheim FA. New York: Oxford University Press; 2006:55-75 [2].

Hormones

Females are affected by RA at a ratio of approximately 3:1 compared with males and experience clinical fluctuations during the menstrual cycle and pregnancy, indicating a major modulating role for sex hormones. Due to their expression of sex-hormone receptors and their cytokine response upon exposure to estrogens, monocytes/Mϕ are strongly involved in hormone modulation of RA [33]. Indeed, physiological levels of estrogens stimulate RA Mϕ to the production of the pro-inflammatory cytokine IL-1, whereas higher levels inhibit IL-1 production, conceivably mimicking the clinical improvement during pregnancy. Interestingly, selective estrogen receptor ligands inhibiting nuclear factor (NF)-κB transcriptional activity (but lacking estrogenic activity) can markedly inhibit joint swelling and destruction in experimental arthritis [34].

Cytokine stimuli with regulatory effects on macrophages

In addition to pro-inflammatory cytokines, several cytokines that regulate monocyte/Mϕ function in RA have been described (summarized in the upper left quadrant of Figure 2). A systematic list of these cytokines is provided in Table 5. Interestingly, some of these molecules are produced by Mϕ themselves (most notably, IL-10), so that autocrine regulation may also play a prominent role during the different clinical phases of RA. Other regulatory cytokines derive from other cell types present in the inflamed synovial membrane: T cells (for example, IL-4 and IL-13) or stromal cells (for example, IL-11). For these molecules, the reader is referred to recent publications or comprehensive reviews [25,35,36].

Monocyte/macrophage effector molecules in rheumatoid arthritis

Monocyte/macrophage effector molecules with proinflammatory effects in rheumatoid arthritis

Mϕ produce a number of pro-inflammatory cytokines, as schematically shown in the upper right quadrant of Figure 2. A systematic list of the pro-inflammatory ILs is provided in Table 4.

Tumor necrosis factor-alpha

TNF-α is a pleiotropic cytokine that increases the expression of cytokines, adhesion molecules, prostaglandin E_2, collagenase, and collagen by synovial cells. TNF-α exists in membrane-bound and soluble forms, both acting as pro-inflammatory mediators. Transmembrane TNF-α is involved in local, cell contact-mediated processes and appears to be the prime stimulator of the R75 receptor [37]. Interestingly, the transgenic expression of this form is alone sufficient to induce chronic arthritis [38]; likewise, a mutant membrane TNF-α, which uses both R55 and R75 receptors, can cause arthritis. Conversely, the soluble form of TNF-α, shed via MMP cleavage from the membrane-bound form, primarily stimulates the R55 receptor, acting transiently and at a distance [37].

In RA, TNF-α is mostly produced by Mϕ in the synovial membrane and at the cartilage-pannus junction and possibly occupies a proximal position in the RA inflammatory cascade [39]. While an average of approximately 5% of synovial cells express TNF-α mRNA/protein *in situ* [40], the degree of TNF-α expression in the synovial tissue depends upon the prevailing histological configuration, resulting in different clinical variants [41]. Different disease stages and clinical variants are also reflected in serum and synovial fluid levels of TNF-α [42].

The critical importance of TNF-α in RA is supported by several experimental observations: (a) TNF-α in combination with IL-1 is a potent inducer of synovitis [43], (b) transgenic, deregulated expression of TNF-α causes the development of chronic arthritis [44], (c) TNF-α is produced in synovial membrane and extra-articular/lymphoid organs in experimental arthritides, mimicking the systemic character of RA [2], (d) neutralization of TNF-α suppresses experimental arthritides [39,43], and (e) administration of chimeric/humanized anti-TNF-α monoclonal antibodies or TNF-α receptor constructs has shown remarkable efficacy in acute disease and retardation of radiographic progression [3,7,11].

As an interesting development, the analysis of gene expression in monocytes of anti-TNF-α-treated patients with RA may represent a powerful tool to identify regulation patterns applicable for diagnosis and therapy stratification or monitoring [45,46] (Stuhlmuller B, Hernandez MM, Haeupl T, Kuban RJ, Gruetzkau A, Voss JW, Salfeld J, Kinne RW, Burmester GR, unpublished data). A reasonable expectation is that gene analyses also provide means to predict which patients are future responders to anti-TNF-α therapy.

Tumor necrosis factor-alpha receptors

TNF receptors are found in synovial tissue and fluid of patients with RA, especially in cases of severe disease [39]. There are two known TNF receptors, the R55 (TNF-R1) (high-affinity receptor) and the R75 (TNF-R2) (low-affinity receptor), which are expressed by both synovial Mϕ and fibroblasts [47,48]. The two TNF receptors can operate independently of one another, cooperatively, or by 'passing' TNF-α to one another [37], a complexity that may explain the tremendous sensitivity of target cells (such as Mϕ) to minute concentrations of TNF-α. TNF receptors can also be shed, binding to soluble TNF-α and hence acting as natural inhibitors in disease. Recent studies have demonstrated that TNF-R1 may be primarily responsible for pro-inflammatory effects of TNF-α, whereas TNF-R2 may predominantly mediate anti-inflammatory effects of TNF-α [48] (reviewed in [49]). Thus, selective blockade of TNF-R1, instead of broad blockade of all effects of TNF-α, may become an attractive therapeutic approach [48,50].

Interleukin-1

In the RA synovial membrane, IL-1 is found predominantly in $CD14^+$ Mϕ [51]; also, IL-1 levels in the synovial fluid significantly correlate with joint inflammation [52]. The two existing forms of IL-1 (IL-1α and IL-1β) show some differ-

Table 5

Overview of anti-inflammatory cytokines relevant to macrophage (dys)function in rheumatoid arthritis

	Anti-inflammatory	Dual	Autocrine	Main pathogenetic features
IL-1RA	X	-	X	Produced by differentiated Mϕ and upregulated by pro-inflammatory mediators, including IL-1 itself or granulocyte macrophage colony-stimulating factor Autocrine contribution to the termination of inflammatory reactions [54,55] (reviewed in [53,56])
IL-4	X	-	-	Strong regulator of Mϕ functions but virtually absent in synovial tissue [73,131-133]
IL-10	X	-	X	Produced by synovial Mϕ Strong regulator of Mϕ functions but relatively deficient in RA Possesses autocrine features [73,74]
IL-11	X	X	-	Regulator of Mϕ functions in a paracrine regulatory loop with synovial fibroblasts [36,134]
IL-13	X	X	-	Selective regulator of Mϕ functions Improves experimental arthritis (reviewed in [2,91])
IL-16	X	X	-	Known as an anti-inflammatory molecule [135,136], IL-16 also has pro-inflammatory properties (that is, correlates with metalloprotease-3 levels, progression of joint destruction, and levels of other pro-inflammatory cytokines) [137,138].
IFN-β	X	-	-	Clear anti-inflammatory and anti-destructive effects in experimental arthritides Therapy attempts in human RA thus far have been unsuccessful [149].
TGF-β	X	X	X	Produced by Mϕ [78-80] Main regulator of connective tissue remodelling Potent inducer of hyaluronan synthase 1 Induces synovial inflammation (reviewed in [80]) but also suppresses acute and chronic arthritis [81,82] Induces inflammation and cartilage degradation in a rabbit model [140] Possesses autocrine features MMP can affect TGF-β via shedding of latent TGF-β attached to decorin (disease-enhancing loop).

IFN-β, interferon-beta; IL, interleukin; IL-1RA, interleukin-1 receptor antagonist; Mϕ, macrophage(s); RA, rheumatoid arthritis; TGF-β, transforming growth factor-beta. Reproduced with permission from Kinne RW, Stuhlmuller B, Palombo-Kinne E, Burmester GR: The role of macrophages in rheumatoid arthritis. In *Rheumatoid Arthritis*. Edited by Firestein GS, Panayi GS, Wollheim FA. New York: Oxford University Press; 2006:55-75 [2].

ences (for example, low protein homology, stronger pro-inflammatory regulation of the IL-1β promoter, and secretion of inactive pro-IL-1β versus expression of membrane-bound IL-1α activity) but also strong similarities (that is, three-dimensional structures of the essential domains, molecular masses of pro-peptides, and mature-form processing proteases), resulting in almost identical binding capacity to the IL-1 receptors and comparable function. In arthritis, IL-1 appears to mediate a large part of the articular damage, as it profoundly influences proteoglycan synthesis and degradation [43,53]. At the same time, IL-1 induces the production of MMP-1 and MMP-3 and enhances bone resorption; this is compatible with recent evidence from arthritis models and human RA suggesting that the tissue-destruction capacities of IL-1β may outweigh its genuine role in joint inflammation [53].

Interleukin-1 receptors

The IL-1 type I receptor (IL-1R1), which mediates cell activation via IL-1R accessory protein and IL-1 receptor-associated kinase (IRAK), is found on numerous cells in the synovial tissue of patients with RA [54]. In contrast, the type II receptor (IL-1R2) (also found in soluble form in serum), which lacks cell-activating properties and acts exclusively as a decoy receptor, is low in synovial tissue [55]. Similarly, IL-1RA, a soluble protein that blocks the action of IL-1 by binding to the type I receptor without receptor activation, has been detected only sporadically in RA synovial samples. In RA, the balance between IL-1 and its physiological inhibitor IL-1RA is therefore shifted in favor of IL-1, indicating a dysregulation crucial in promoting chronicity [53]. However, therapeutic application of IL-1RA (anakinra) appears to be only modestly effective in RA (reviewed in [56]). Therefore, it remains to be clarified whether the IL-1 pathway is a less suitable therapeutic target than TNF-α (for example, due to functional redundancy in the IL-1 receptor superfamily) or whether the biological molecule IL-1RA is suboptimal for therapy.

Interleukin-15

IL-15, a cytokine of the IL-2 family with chemoattractant properties for memory T cells, is produced by lining layer cells

(including Mϕ) and is increased in RA synovial fluid [57]. Notably, peripheral or synovial T cells stimulated with IL-15 induce Mϕ to produce IL-1β, TNF-α, IL-8, and monocyte chemotactic protein-1 [21,57] but not the regulatory IL-10. Because IL-15 is also produced by Mϕ themselves, this cytokine may (re)stimulate T cells, possibly self-perpetuating a pro-inflammatory loop [57]. The expression of IL-15 in the RA synovial membrane, its biological function, and its successful targeting in experimental arthritis have generated large expectations on the use of a fully humanized anti-IL-15 antibody in clinical trials [56-58].

Interleukin-18

In the RA synovial membrane, this cytokine of the IL-1 family is expressed in $CD68^+$ Mϕ contained in lymphoid aggregates. $CD14^+$ Mϕ of the RA synovial fluid also express the IL-18 receptor [59]. The pro-inflammatory role of IL-18 in arthritis (and its potential suitability as a therapeutic target in RA) is indicated by the following findings: (a) IL-18 treatment markedly aggravates experimental arthritis [59], (b) intra-articular overexpression of IL-18 induces experimental arthritis, (c) IL-18 is involved in the development of experimental streptococcal arthritis (a strongly Mϕ-dependent model), (d) IL-18 is selectively overexpressed in the bone marrow of patients with juvenile idiopathic arthritis and Mϕ activation syndrome [5], (e) IL-18 can stimulate osteoclast formation through upregulation of RANKL (receptor activator of NF-κB ligand) production by T cells in RA synovitis, and (f) IL-18 mediates its action via classic induction of TNF-α, GM-CSF, and interferon (IFN)-γ [59] or functional Toll-like receptors TLR-2 and TLR-4 in synovial cells [30] or else through the induction of synovial acute-phase serum amyloid proteins. The clinical relevance of synovial IL-18 is emphasised by its correlation with the systemic levels of C-reactive protein (CRP); also, IL-18 and CRP decrease in parallel in synovial tissue and serum following effective treatment with disease-modifying anti-rheumatic drugs [60]. In addition, peripheral blood mononuclear cells of RA patients show low levels of the IL-18 binding protein (a natural inhibitor of IL-18) and reduced sensitivity to stimulation with IL-12/IL-18, indicating profound dysregulation of the IL-18 system [61].

Interleukin-23

The genuine role of IL-23, a cytokine of the IL-12 family predominantly produced by Mϕ or dendritic cells, is unclear due to the sharing of the p40 subunit with IL-12 [62]. IL-23 has prominent pro-inflammatory functions, since transgenic expression in mice leads to multi-organ inflammation and premature death. IL-23 promotes various T-cell responses potentially relevant to RA [62]. Recent studies in experimental arthritis have demonstrated that mice lacking only IL-12 ($p35^{-/-}$) show exacerbated arthritis, whereas mice lacking only IL-23 ($p19^{-/-}$) are completely protected from arthritis [63]. In addition, activation of Mϕ derived from arthritis-susceptible rats is paradoxically associated with reduced levels of pro-inflammatory mediators but high expression of IL-23 (p19), whereas non-susceptible rats show the inverse phenotype. If these findings were transferable to human RA, IL-23 would have a pro-inflammatory role and IL-12 a protective one. At the present time, it is unclear whether these findings fit into the recently introduced M1/M2 paradigm of differential Mϕ activation [64,65] and especially whether this paradigm can be exploited for a better understanding of the role of Mϕ in RA.

Interleukin-27

IL-27, another cytokine of the IL-12 family, is expressed by monocytes/Mϕ following common inflammatory stimuli and displays a variety of pro- and anti-inflammatory properties [66]. In support of a pro-inflammatory role in arthritis, neutralizing antibodies against IL-27p28 suppress experimental arthritis [67].

Chemokines and chemokine receptors

Chemokines (subdivided into the CXC, CC, C, and CX3C families) are small proteins specialized in differential recruitment of leukocyte populations via a number of transmembrane receptors. Chemokines not only favor monocyte influx into inflamed tissue, but also play a key role in activation, functional polarization, and homing of patrolling monocytes/Mϕ [65]. Notably, monocytes/Mϕ express only select types of the numerous chemokine receptors (for example, CCR1, 2, 5, 7, and 8 as well as CX3CR1), representing a partially specific basis for prominent trafficking of monocyte/Mϕ in arthritis. In RA, synovial Mϕ produce several chemokines (for example, CCL3 [or Mϕ inflammatory protein 1α], CCL5 [or RANTES], and CX3CL1 [or fractalkine]) and at the same time carry chemokine receptors, indicating the presence of autocrine loops in disease (reviewed in [68]). At the same time, chemokines are upregulated by the Mϕ-derived TNF-α and IL-1. Significantly, some chemokines expressed in synovial Mϕ (for example, IL-8 and fractalkine) are powerful promoters of angiogenesis, thus providing a link between Mϕ activation and the prominent neo-vascularization of the RA synovium [69]. In RA, angiogenesis may be further promoted via activation of Mϕ by advanced glycation end products, whereas thrombospondin-2 seems to downregulate angiogenesis. Because the enlargement of the vascular bed potentiates the influx of activated monocytes, down-modulation of the chemokine system represents a multi-potential target of anti-rheumatic therapy, as indicated by the promising results of treatment with a CCR1 antagonist in RA [68].

Macrophage migration inhibitory factor

One of the first ILs ever discovered, migration inhibitory factor (MIF), is an early-response cytokine abundantly released by Mϕ. MIF stimulates a number of Mϕ functions in an autocrine fashion (for example, secretion of TNF-α, phagocytosis, and generation of reactive oxygen species [ROS]). In addition, MIF confers resistance to apoptosis in Mϕ and synovial fibroblasts, thus prolonging the survival of activated, disease-

relevant cells. In RA, MIF is overexpressed in serum and synovial tissue in correlation with disease activity. Also, polymorphisms in the promoter or coding region of the human MIF gene are associated with features of juvenile idiopathic arthritis or adult RA [70].

Monocyte/macrophage effector molecules with anti-inflammatory/regulatory effects in rheumatoid arthritis

Mϕ also produce anti-inflammatory cytokines, most notably IL-RA and IL-10, both cytokines engaged in autocrine regulatory loops (shown in the lower right quadrant of Figure 2) (Table 5).

Interleukin-1 receptor antagonist

Differentiated Mϕ constitutively express IL-1RA, which is upregulated by pro-inflammatory mediators, including IL-1 itself or GM-CSF, and induces strong anti-inflammatory effects. By means of this feedback mechanism, Mϕ therefore contribute to the termination of inflammatory reactions (reviewed in [71,72]) (see above).

Interleukin-10

IL-10, a Th_2- and Mϕ-derived cytokine with clear autocrine functions, reduces HLA-DR expression and antigen presentation in monocytes and inhibits the production of pro-inflammatory cytokines, GM-CSF, and Fc-γ receptors by synovial Mϕ. Consistently with cytokine and chemokine downregulation, IL-10 clearly suppresses experimental arthritis. In spite of IL-10 elevation in serum and synovial compartments of patients with RA [73], some studies suggest a relative deficiency of IL-10 [74]. A combined IL-4/IL-10 deficiency probably tilts the cytokine balance to a pro-inflammatory predominance. In addition, the *ex vivo* production of IL-10 by RA peripheral blood mononuclear cells is negatively correlated with radiographic joint damage and progression of joint damage, suggesting that high IL-10 production is protective in RA. Similarly to IL-4, however, treatment with recombinant IL-10 does not improve RA. This may be partially explained by upregulation of the pro-inflammatory Fc-γ receptors I and IIA on monocytes/Mϕ (reviewed in [2]).

Monocyte/macrophage effector molecules with dual effects in rheumatoid arthritis

Cytokines with a dual role are indicated in Tables 4 and 5.

Interleukin-6

IL-6 is the most strikingly elevated cytokine in RA, especially in the synovial fluid during acute disease [75]. The acute rise is consistent with the role of IL-6 in acute-phase responses (Table 1). However, while IL-6 levels in the synovial fluid correlate with the degree of radiological joint damage, and IL-6 and soluble IL-6 receptors promote the generation of osteoclasts, this cytokine has phase-dependent effects; for example, it protects cartilage in acute disease but promotes excessive bone formation in chronic disease. While IL-6 is mostly produced by synovial fibroblasts and only partially by Mϕ, two findings suggest that the striking IL-6 rise is a prominent outcome of Mϕ activation: (a) the morphological vicinity of IL-6-expressing fibroblasts with $CD14^+$ Mϕ in the RA synovial tissue (reviewed in [2]) and (b) co-culture studies showing that IL-1 stimulates IL-6 production [17]. The role of IL-6 in experimental arthritis and the anti-arthritic effects of anti-IL-6 receptor antibodies suggest a role for anti-IL-6 therapy in RA [76] (reviewed in [77]).

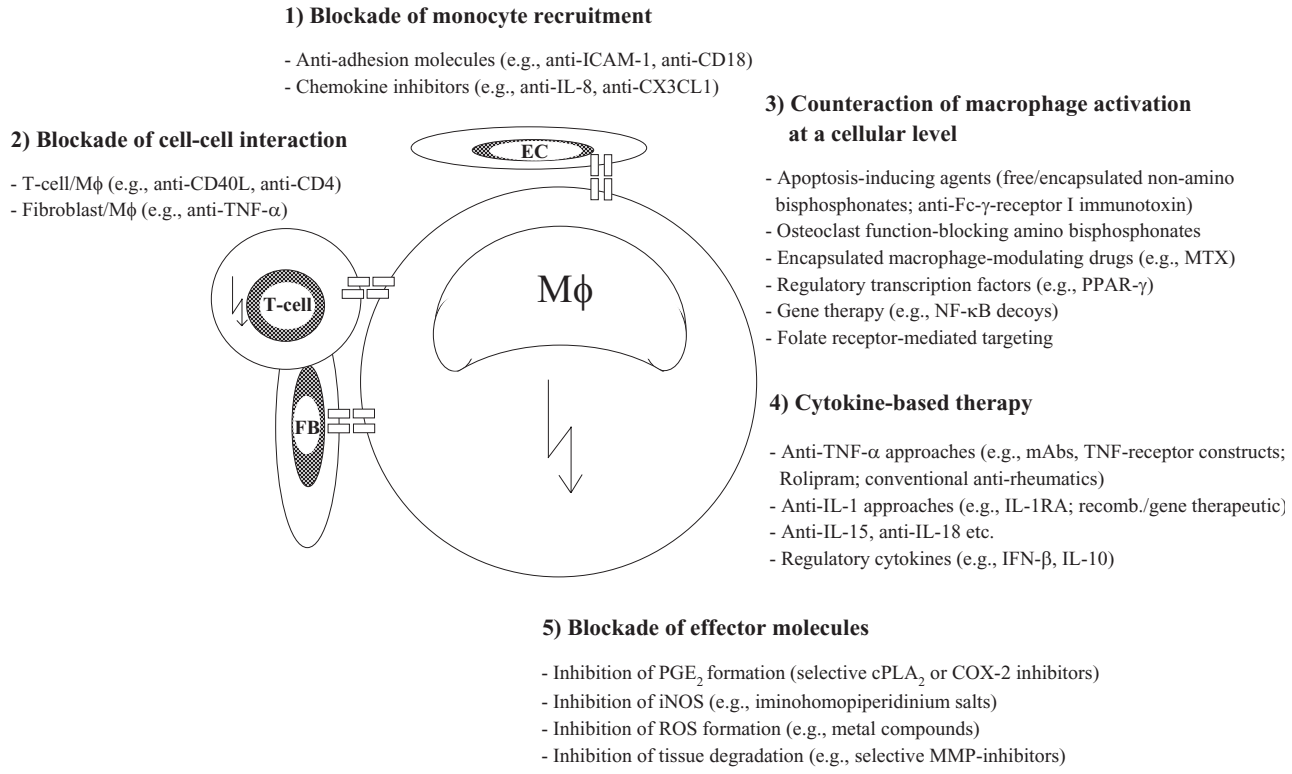
Transforming growth factor-beta

In RA, Mϕ express different transforming growth factor-beta (TGF-β) molecules and TGF-β receptors in the lining and sublining layers, at the cartilage-pannus junction, and in the synovial fluid [78-80]. The pro-inflammatory effects of TGF-β are substantiated by induction of Mϕ expression of Fc-γ receptor III (which elicits the release of tissue-damaging ROS) and promotion of monocyte adhesion and infiltration during chronic disease (reviewed in [80]). At the same time, TGF-β has anti-inflammatory properties; for example, it counteracts some IL-1 effects, including phagocytosis of collagen and possibly MMP production. A protective role of TGF-β in RA is also suggested by the association between TGF-β polymorphism and disease severity; that is, alleles associated with low TGF-β expression are correlated with stronger inflammation and poorer outcome [81]. Likewise, experimental arthritis is significantly ameliorated by activation of TGF-β via adenoviral expression of thrombospondin-1 [82]. The effects of TGF-β on tissue inhibitor of MMP (TIMP) are also unclear, as the regulation of MMP and TIMP may depend on different tissue domains (superficial versus deep cartilage layers) and may vary for intra- or extracellular digestion of collagen (reviewed in [2]).

Treatment of human rheumatoid arthritis with conventional anti-macrophage approaches

The role of Mϕ-derived cytokines in the perpetuation of RA, the pathophysiological dichotomy between joint inflammation and cartilage destruction, and the crucial significance of activated synovial Mϕ in relation to permanent joint damage [1] have led to a radical re-evaluation of the conventional anti-inflammatory and disease-modifying treatments in relation to Mϕ parameters in order to potentiate therapeutic effects (for example, via combination approaches [83]) and reduce side effects. For anti-Mϕ effects of conventional anti-rheumatic therapy in RA (including methotrexate, leflunomide, anti-malarials, gold compounds, corticosteroids, and non-steroidal anti-inflammatory drugs), the reader is referred to a recent comprehensive review [11]. Recent findings show that conventional and specific anti-rheumatic treatments predominantly target sublining rather than lining Mϕ; also, different therapeutic approaches seem to result in similar histological changes in the inflamed synovial membrane, including significant reduction of sublining Mϕ. This, in turn, is significantly correlated with the degree of clinical improvement [11,12]. Thus, different pathogenetic mechanisms may funnel into

Figure 3



Potential and established approaches for modulation of monocyte/macrophage (Mϕ) functions in rheumatoid arthritis. COX-2, cyclooxygenase-2; EC, endothelial cells; FB, fibroblasts; ICAM-1, intracellular adhesion molecule-1; IFN-β, interferon-beta; IL, interleukin; IL-1RA, interleukin-1 receptor antagonist; iNOS, inducible nitric-oxide synthase; mAbs, mononuclear antibodies; $cPLA_2$, cytosolic phospholipase A_2; MMP, metalloprotease; MTX, methotrexate; NF-κB, nuclear factor-kappa-B; PGE_2, prostaglandin E_2; PPAR-γ, peroxisome proliferator-activated receptor-gamma; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-alpha. Reproduced with permission from Kinne RW, Stuhlmuller B, Palombo-Kinne E, Burmester GR: The role of macrophages in rheumatoid arthritis. In *Rheumatoid Arthritis*. Edited by Firestein GS, Panayi GS, Wollheim FA. New York: Oxford University Press; 2006:55-75 [2].

similar disease pathway(s), leading to massive activation of Mϕ and providing the rationale for targeted anti-Mϕ therapy.

Non-conventional and experimental anti-macrophage therapy

Counteraction of monocyte/macrophage activation at a cellular level

Apoptosis-inducing agents

Physical elimination of disease-relevant cells (for example, activated Mϕ or osteoclasts) by apoptosis is advantageous because it circumvents secondary tissue damage by restraining cellular organelles in apoptotic vesicles. Phagocytic incorporation of liposome-encapsulated non-amino-bisphosphonates by activated monocytes, for example, induces apoptosis in these cells [84] (Figure 3). Systemic application of encapsulated bisphosphonates in experimental arthritis not only counteracts joint swelling, but also prevents local joint destruction and subchondral bone damage [85]; in addition, it shows protective effects on remote bone damage. Studies in RA show that a single intra-articular administration of clodronate liposomes leads to Mϕ depletion and decreased expression of adhesion molecules in the lining layer of RA synovial tissue [86]. Selective targeting of activated Mϕ has also been demonstrated using either apoptosis-inducing immunotoxins coupled to anti-Fc-γ receptor I (CD64) antibodies or folate receptor-mediated targeting (reviewed in [2]). In general, liposome encapsulation can also be exploited for selective delivery of Mϕ-modulating drugs [87] (reviewed in [2]) or gene therapy constructs (reviewed in [88]).

Control of gene transcription

The transcription of most cytokine genes in monocytes/Mϕ depends on the activation of NF-κB and NF-κM transcription factors or that of the activator protein-1 (AP-1) complex. In RA synovial Mϕ, the expression of NF-κB is more pronounced than that of AP-1, a selectivity that may bear important therapeutic implications [89]. Accordingly, the anti-arthritic effects of IL-4 may be based on the selective suppression of NF-κB in Mϕ. IL-10 also downregulates the production of pro-inflammatory monokines, inhibiting the nuclear factors NF-κB,

AP-1, or NF-IL-6. Unlike IL-4, IL-10 can also enhance degradation of the mRNA for IL-1 and TNF-α (reviewed in [2]). In general, therefore, targeted inhibition of 'pro-inflammatory' signal transduction pathways in Mϕ represents an attractive therapeutic approach [90].

Gene therapy in experimental arthritis

Gene therapy has been applied in experimental arthritis models to counteract Mϕ-derived IL-1 and TNF-α or to deliver/overexpress protective IL-1RA, soluble IL-1 type I receptor-IgG fusion protein, and type I soluble TNF-α receptor-IgG fusion protein. This has been extended to (Mϕ-derived) anti-inflammatory cytokines (that is, IL-4, IL-10, IL-13, IFN-β, or TGF-β) and to 'molecular synovectomy' (either by expression of herpes simplex virus-thymidine kinase with subsequent administration of ganciclovir or by overexpression of Fas-ligand/inhibitors of nuclear translocation of NF-κB, resulting in synovial cell apoptosis [88,91,92]). Therefore, gene therapy aimed at neutralizing pro-inflammatory Mϕ products, overexpressing Mϕ-regulating mediators, or simply eliminating overly activated Mϕ remains promising for the treatment of arthritis.

Conclusion

The multitude and abundance of Mϕ-derived mediators in RA and their paracrine and autocrine effects (including those directed to other cells of the myeloid lineage) indicate that Mϕ are local and systemic amplifiers of disease severity and perpetuation. The main local mechanisms include (a) self-perpetuating chemokine-mediated recruitment of inflammatory cells, (b) cytokine-mediated activation of newly immigrated inflammatory cells, (c) cell contact-mediated activation of neighboring inflammatory cells, (d) cytokine- and cell contact-mediated secretion of matrix-degrading enzymes, (e) activation of mature dendritic cells and cytokine-mediated differentiation of Mϕ (and possibly B cells, T cells, and mesenchymal cells) into antigen-presenting cells, with possible effects on spreading of autoimmunity to cryptic epitopes, (f) neovascularization, with potentiation of cellular and exudatory mechanisms, and (g) (trans)differentiation of Mϕ into osteoclasts involved in subchondral bone damage. At a systemic level, amplification of disease can proceed at least through the following mechanisms: (a) acute-phase response network, (b) systemic production of TNF-α, (c) anomalies in bone marrow differentiation, and (d) chronic activation of circulating monocytes.

Although uncovering the etiology of disease remains the ultimate goal of research, the efforts in understanding how activated Mϕ influence disease have led to optimization strategies to selectively target activated Mϕ in RA (Figure 3). This approach has at least two advantages: (a) striking the very cell population that mediates/amplifies most of the irreversible cartilage destruction and (b) minimizing adverse effects on other cells that may have no (or marginal) effects on joint damage.

This review is part of a series on
Cells of the synovium in rheumatoid arthritis
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Competing interests

The authors declare that they have no competing interests.

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References

1. Mulherin D, Fitzgerald O, Bresnihan B: **Synovial tissue macrophage populations and articular damage in rheumatoid arthritis.** *Arthritis Rheum* 1996, **39:**115-124.
2. Kinne RW, Stuhlmuller B, Palombo-Kinne E, Burmester GR: **The role of macrophages in rheumatoid arthritis.** In *Rheumatoid Arthritis*. Edited by Firestein GS, Panayi GS, Wollheim FA. New York: Oxford University Press; 2006:55-75.
3. Smolen JS, Steiner G: **Therapeutic strategies for rheumatoid arthritis.** *Nat Rev Drug Discov* 2003, **2:**473-488.
4. Stuhlmüller B, Ungethüm U, Scholze S, Martinez L, Backhaus M, Kraetsch HG, Kinne RW, Burmester GR: **Identification of known and novel genes in activated monocytes from patients with rheumatoid arthritis.** *Arthritis Rheum* 2000, **43:**775-790.
5. Maeno N, Takei S, Imanaka H, Yamamoto K, Kuriwaki K, Kawano Y, Oda H: **Increased interleukin-18 expression in bone marrow of a patient with systemic juvenile idiopathic arthritis and unrecognized macrophage-activation syndrome.** *Arthritis Rheum* 2004, **50:**1935-1938.
6. Lavagno L, Gunella G, Bardelli C, Spina S, Fresu LG, Viano I, Brunelleschi S: **Anti-inflammatory drugs and tumor necrosis factor-alpha production from monocytes: role of transcription factor NF-kappaB and implication for rheumatoid arthritis therapy.** *Eur J Pharmacol* 2004, **501:**199-208.
7. Feldmann M, Brennan FM, Foxwell BM, Taylor PC, Williams RO, Maini RN: **Anti-TNF therapy: where have we got to in 2005?** *J Autoimmun* 2005, **25 Suppl:**26-28.
8. van Rooijen N, Kesteren-Hendrikx E: **'*In vivo*' depletion of macrophages by liposome-mediated 'suicide'.** *Methods Enzymol* 2003, **373:**3-16.
9. Sweeney SE, Firestein GS: **Signal transduction in rheumatoid arthritis.** *Curr Opin Rheumatol* 2004, **16:**231-237.
10. Westra J, Doornbos-van der Meer B, de Boer P, van Leeuwen MA, van Rijswijk MH, Limburg PC: **Strong inhibition of TNF-alpha production and inhibition of IL-8 and COX-2 mRNA expression in monocyte-derived macrophages by RWJ 67657, a p38 mitogen-activated protein kinase (MAPK) inhibitor.** *Arthritis Res Ther* 2004, **6:**R384-R392.
11. Franz JK, Burmester GR: **The needle and the damage done.** *Ann Rheum Dis* 2005, **64:**798-800.
12. Haringman JJ, Gerlag DM, Zwinderman AH, Smeets TJ, Kraan MC, Baeten D, McInnes IB, Bresnihan B, Tak PP: **Synovial tissue macrophages: a sensitive biomarker for response to treatment in patients with rheumatoid arthritis.** *Ann Rheum Dis* 2005, **64:**834-838.

13. Gordon S, Taylor PR: **Monocyte and macrophage heterogeneity.** *Nat Rev Immunol* 2005, **5:**953-964.
14. Sattar N, McCarey DW, Capell H, McInnes IB: **Explaining how 'high-grade' systemic inflammation accelerates vascular risk in rheumatoid arthritis.** *Circulation* 2003, **108:**2957-2963.
15. Monaco C, Andreakos E, Kiriakidis S, Feldmann M, Paleolog E: **T-cell-mediated signalling in immune, inflammatory and angiogenic processes: the cascade of events leading to inflammatory diseases.** *Curr Drug Targets Inflamm Allergy* 2004, **3:**35-42.
16. Kinne RW, Stuhlmuller B, Palombo-Kinne E, Burmester GR: **The role of macrophages in the pathogenesis of rheumatoid arthritis.** In *Rheumatoid Arthritis: The New Frontiers in Pathogenesis and Treatment*. Edited by Wollheim F, Firestein GS, Panayi GS. Oxford: Oxford University Press; 2000:69-87.
17. Chomarat P, Rissoan MC, Pin JJ, Banchereau J, Miossec P: **Contribution of IL-1, CD14, and CD13 in the increased IL-6 production induced by *in vitro* monocyte-synoviocyte interactions.** *J Immunol* 1995, **155:**3645-3652.
18. Scott BB, Weisbrot LM, Greenwood JD, Bogoch ER, Paige CJ, Keystone EC: **Rheumatoid arthritis synovial fibroblast and U937 macrophage/monocyte cell line interaction in cartilage degradation.** *Arthritis Rheum* 1997, **40:**490-498.
19. McInnes IB, Leung BP, Liew FY: **Cell-cell interactions in synovitis: Interactions between T lymphocytes and synovial cells.** *Arthritis Res* 2000, **2:**374-378.
20. Burger D, Dayer JM: **The role of human T-lymphocyte-monocyte contact in inflammation and tissue destruction.** *Arthritis Res* 2002, **4:**S169-S176.
21. Sebbag M, Parry SL, Brennan FM, Feldmann M: **Cytokine stimulation of T lymphocytes regulates their capacity to induce monocyte production of tumor necrosis factor-alpha, but not interleukin-10: possible relevance to pathophysiology of rheumatoid arthritis.** *Eur J Immunol* 1997, **27:**624-632.
22. Tran CN, Lundy SK, Fox DA: **Synovial biology and T cells in rheumatoid arthritis.** *Pathophysiology* 2005, **12:**183-189.
23. Dalbeth N, Gundle R, Davies RJ, Lee YC, McMichael AJ, Callan MF: **CD56bright NK cells are enriched at inflammatory sites and can engage with monocytes in a reciprocal program of activation.** *J Immunol* 2004, **173:**6418-6426.
24. Miossec P: **An update on the cytokine network in rheumatoid arthritis.** *Curr Opin Rheumatol* 2004, **16:**218-222.
25. Lundy SK, Sarkar S, Tesmer LA, Fox DA: **Cells of the synovium in rheumatoid arthritis. T lymphocytes.** *Arthritis Res Ther* 2007, **9:**202.
26. Giles JT, Bathon JM: **Serious infections associated with anticytokine therapies in the rheumatic diseases.** *J Intensive Care Med* 2004, **19:**320-334.
27. Gartlehner G, Hansen RA, Jonas BL, Thieda P, Lohr KN: **The comparative efficacy and safety of biologics for the treatment of rheumatoid arthritis: a systematic review and metaanalysis.** *J Rheumatol* 2006, **33:**2398-2408.
28. Hopkins PA, Fraser JD, Pridmore AC, Russell HH, Read RC, Sriskandan S: **Superantigen recognition by HLA class II on monocytes up-regulates toll-like receptor 4 and enhances proinflammatory responses to endotoxin.** *Blood* 2005, **105:** 3655-3662.
29. Seibl R, Kyburz D, Lauener RP, Gay S: **Pattern recognition receptors and their involvement in the pathogenesis of arthritis.** *Curr Opin Rheumatol* 2004, **16:**411-418.
30. Pierer M, Rethage J, Seibl R, Lauener R, Brentano F, Wagner U, Hantzschel H, Michel BA, Gay RE, Gay S, *et al.*: **Chemokine secretion of rheumatoid arthritis synovial fibroblasts stimulated by Toll-like receptor 2 ligands.** *J Immunol* 2004, **172:** 1256-1265.
31. Frasnelli ME, Tarussio D, Chobaz-Peclat V, Busso N, So A: **TLR2 modulates inflammation in zymosan-induced arthritis in mice.** *Arthritis Res Ther* 2005, **7:**R370-R379.
32. Kawane K, Ohtani M, Miwa K, Kizawa T, Kanbara Y, Yoshioka Y, Yoshikawa H, Nagata S: **Chronic polyarthritis caused by mammalian DNA that escapes from degradation in macrophages.** *Nature* 2006, **443:**998-1002.
33. Cutolo M, Lahita RG: **Estrogens and arthritis.** *Rheum Dis Clin North Am* 2005, **31:**19-27.
34. Keith JC, Albert LM, Leathurby Y, Follettie M, Wang L, Borges-Marcucci L, Chadwick CC, Steffan RJ, Harnish DC: **The utility of pathway selective estrogen receptor ligands that inhibit nuclear factor-kB transcriptional activity in models of rheumatoid arthritis.** *Arthritis Res Ther* 2005, **7:**R427-R438.
35. Taylor PC: **Anti-cytokines and cytokines in the treatment of rheumatoid arthritis.** *Curr Pharm Des* 2003, **9:**1095-1106.
36. Wong PK, Campbell IK, Robb L, Wicks IP: **Endogenous IL-11 is pro-inflammatory in acute methylated bovine serum albumin/interleukin-1-induced (mBSA/IL-1)arthritis.** *Cytokine* 2005, **29:**72-76.
37. Grell M, Douni E, Wajant H, Löhden M, Clauss M, Maxeiner B, Georgopoulos S, Lesslauer W, Kollias G, Pfizenmaier K, *et al.*: **The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor.** *Cell* 1995, **83:**793-802.
38. Georgopoulos S, Plows D, Kollias G: **Transmembrane TNF is sufficient to induce localized tissue toxicity and chronic inflammatory arthritis in transgenic mice.** *J Inflamm* 1996, **46:**86-97.
39. Feldmann M, Brennan FM, Maini RN: **Role of cytokines in rheumatoid arthritis.** *Annu Rev Immunol* 1996, **14:**397-440.
40. Firestein GS, Alvaro-Gracia JM, Maki R, Alvaro-Garcia JM: **Quantitative analysis of cytokine gene expression in rheumatoid arthritis.** *J Immunol* 1990, **144:**3347-3353.
41. Klimiuk PA, Goronzy JJ, Björ nsson J, Beckenbaugh RD, Weyand CM: **Tissue cytokine patterns distinguish variants of rheumatoid synovitis.** *Am J Pathol* 1997, **151:**1311-1319.
42. Klimiuk PA, Sierakowski S, Latosiewicz R, Cylwik B, Skowronski J, Chwiecko J: **Serum cytokines in different histological variants of rheumatoid arthritis.** *J Rheumatol* 2001, **28:**1211-1217.
43. van den Berg WB, Joosten LA, Kollias G, Van De Loo FA: **Role of tumour necrosis factor alpha in experimental arthritis: separate activity of interleukin 1beta in chronicity and cartilage destruction.** *Ann Rheum Dis* 1999, **58:**I40-I48.
44. Kollias G: **Modeling the function of tumor necrosis factor in immune pathophysiology.** *Autoimmun Rev* 2004, **3:**S24-S25.
45. Toh ML, Marotte H, Blond JL, Jhumka U, Eljaafari A, Mougin B, Miossec P: **Overexpression of synoviolin in peripheral blood and synoviocytes from rheumatoid arthritis patients and continued elevation in nonresponders to infliximab treatment.** *Arthritis Rheum* 2006, **54:**2109-2118.
46. Lequerré T, Gauthier-Jauneau AC, Bansard C, Derambure C, Hiron M, Vittecoq O, Daveau M, Mejjad O, Daragon A, Tron F, *et al.*: **Gene profiling in white blood cells predicts infliximab responsiveness in rheumatoid arthritis.** *Arthritis Res Ther* 2006, **8:**R105.
47. Alsalameh S, Winter K, Al-Ward R, Wendler J, Kalden JR, Kinne RW: **Distribution of TNF-alpha, TNF-R55 and TNF-R75 in the rheumatoid synovial membrane: TNF receptors are localized preferentially in the lining layer; TNF-alpha is distributed mainly in the vicinity of TNF receptors in the deeper layers.** *Scand J Immunol* 1999, **49:**278-285.
48. Kunisch E, Gandesiri M, Fuhrmann R, Roth A, Winter R, Kinne RW: **Predominant activation of MAP kinases and pro-destructive/pro-inflammatory features by TNF-alpha in early-passage synovial fibroblasts via tumor necrosis factor receptor- 1: failure of p38 inhibition to suppress matrix metalloproteinase-1 in rheumatoid arthritis.** *Ann Rheum Dis* 2007, **66:**1043-1051.
49. Alsalameh S, Amin RJ, Kunisch E, Jasin HE, Kinne RW: **Preferential induction of prodestructive matrix metalloproteinase-1 and proinflammatory interleukin 6 and prostaglandin E2 in rheumatoid arthritis synovial fibroblasts via tumor necrosis factor receptor-55.** *J Rheumatol* 2003, **30:**1680-1690.
50. Deng GM, Zheng L, Chan FK, Lenardo M: **Amelioration of inflammatory arthritis by targeting the pre-ligand assembly domain of tumor necrosis factor receptors.** *Nat Med* 2005, **11:** 1066-1072.
51. Wood NC, Dickens E, Symons JA, Duff GW: ***In situ* hybridization of interleukin-1 in CD14-positive cells in rheumatoid arthritis.** *Clin Immunol Immunopathol* 1992, **62:**295-300.
52. Arend WP, Malyak M, Guthridge CJ, Gabay C: **Interleukin-1 receptor antagonist: role in biology.** *Annu Rev Immunol* 1998, **16:**27-55.
53. Dinarello CA: **The IL-1 family and inflammatory diseases.** *Clin Exp Rheumatol* 2002, **20:**S1-S13.
54. Deleuran BW, Chu CQ, Field M, Brennan FM, Katsikis P, Feldmann M, Maini RN: **Localization of interleukin-1 alpha, type 1 interleukin-1 receptor and interleukin-1 receptor antagonist in the synovial membrane and cartilage/pannus junction in rheumatoid arthritis.** *Br J Rheumatol* 1992, **31:**801-809.

55. Silvestri T, Pulsatelli L, Dolzani P, Frizziero L, Facchini A, Meliconi R: ***In vivo*** **expression of inflammatory cytokine receptors in the joint compartments of patients with arthritis.** *Rheumatol Int* 2006, **26:**360-368.
56. McInnes IB, Liew FY: **Cytokine networks-towards new therapies for rheumatoid arthritis.** *Nat Clin Pract Rheumatol* 2005, **1:** 31-39.
57. McInnes IB, Gracie JA: **Interleukin-15: a new cytokine target for the treatment of inflammatory diseases.** *Curr Opin Pharmacol* 2004, **4:**392-397.
58. Connell L, McInnes IB: **New cytokine targets in inflammatory rheumatic diseases.** *Best Pract Res Clin Rheumatol* 2006, **20:**865-878.
59. Gracie JA: **Interleukin-18 as a potential target in inflammatory arthritis.** *Clin Exp Immunol* 2004, **136:**402-404.
60. Rooney T, Murphy E, Benito M, Roux-Lombard P, FitzGerald O, Dayer JM, Bresnihan B: **Synovial tissue interleukin-18 expression and the response to treatment in patients with inflammatory arthritis.** *Ann Rheum Dis* 2004, **63:**1393-1398.
61. Dinarello CA: **Interleukin-18 and the pathogenesis of inflammatory diseases.** *Semin Nephrol* 2007, **27:**98-114.
62. Vandenbroeck K, Alloza I, Gadina M, Matthys P: **Inhibiting cytokines of the interleukin-12 family: recent advances and novel challenges.** *J Pharm Pharmacol* 2004, **56:**145-160.
63. Murphy CA, Langrish CL, Chen Y, Blumenschein W, McClanahan T, Kastelein RA, Sedgwick JD, Cua DJ: **Divergent pro- and anti-inflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation.** *J Exp Med* 2003, **198:**1951-1957.
64. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM: **M-1/M-2 macrophages and the Th1/Th2 paradigm.** *J Immunol* 2000, **164:**6166-6173.
65. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M: **The chemokine system in diverse forms of macrophage activation and polarization.** *Trends Immunol* 2004, **25:**677-686.
66. Villarino AV, Hunter CA: **Biology of recently discovered cytokines: discerning the pro- and anti-inflammatory properties of interleukin-27.** *Arthritis Res Ther* 2004, **6:**225-233.
67. Goldberg R, Wildbaum G, Zohar Y, Maor G, Karin N: **Suppression of ongoing adjuvant-induced arthritis by neutralizing the function of the p28 subunit of IL-27.** *J Immunol* 2004, **173:** 1171-1178.
68. Haringman JJ, Kraan MC, Smeets TJ, Zwinderman KH, Tak PP: **Chemokine blockade and chronic inflammatory disease: proof of concept in patients with rheumatoid arthritis.** *Ann Rheum Dis* 2003, **62:**715-721.
69. Koch AE: **Angiogenesis as a target in rheumatoid arthritis.** *Ann Rheum Dis* 2003, **62:**ii60-ii67.
70. Morand EF, Leech M: **Macrophage migration inhibitory factor in rheumatoid arthritis.** *Front Biosci* 2005, **10:**12-22.
71. Bresnihan B: **Anakinra as a new therapeutic option in rheumatoid arthritis: clinical results and perspectives.** *Clin Exp Rheumatol* 2002, **20:**S32-S34.
72. Dinarello CA: **Therapeutic strategies to reduce IL-1 activity in treating local and systemic inflammation.** *Curr Opin Pharmacol* 2004, **4:**378-385.
73. Isomaki P, Luukkainen R, Saario R, Toivanen P, Punnonen J: **Interleukin-10 functions as an antiinflammatory cytokine in rheumatoid synovium.** *Arthritis Rheum* 1996, **39:**386-395.
74. Katsikis PD, Chu CQ, Brennan FM, Maini RN, Feldmann M: **Immunoregulatory role of interleukin 10 in rheumatoid arthritis.** *J Exp Med* 1994, **179:**1517-1527.
75. Houssiau FA, Devogelaer JP, Van Damme J, de Deuxchaisnes CN, Van Snick J: **Interleukin-6 in synovial fluid and serum of patients with rheumatoid arthritis and other inflammatory arthritides.** *Arthritis Rheum* 1988, **31:**784-788.
76. Maini RN, Taylor PC, Szechinski J, Pavelka K, Bröll J, Balint G, Emery P, Raemen F, Petersen J, Smolen J, *et al.*: **Double-blind randomized controlled clinical trial of the interleukin-6 receptor antagonist, tocilizumab, in European patients with rheumatoid arthritis who had an incomplete response to methotrexate.** *Arthritis Rheum* 2006, **54:**2817-2829.
77. Smolen JS, Maini RN: **Interleukin-6: a new therapeutic target.** *Arthritis Res Ther* 2006, **8 Suppl 2:**S5.
78. Chu CQ, Field M, Abney E, Zheng RQ, Allard S, Feldmann M, Maini RN: **Transforming growth factor-beta 1 in rheumatoid synovial membrane and cartilage/pannus junction.** *Clin Exp Immunol* 1991, **86:**380-386.
79. Szekanecz Z, Haines GK, Harlow LA, Shah MR, Fong TW, Fu R, Lin SJ, Rayan G, Koch AE: **Increased synovial expression of transforming growth factor (TGF)-beta receptor endoglin and TGF-beta 1 in rheumatoid arthritis: possible interactions in the pathogenesis of the disease.** *Clin Immunol Immunopathol* 1995, **76:**187-194.
80. Chen W, Wahl SM: **TGF-beta: receptors, signaling pathways and autoimmunity.** *Curr Dir Autoimmun* 2002, **5:**62-91.
81. Mattey DL, Nixon N, Dawes PT, Kerr J: **Association of polymorphism in the transforming growth factor {beta}1 gene with disease outcome and mortality in rheumatoid arthritis.** *Ann Rheum Dis* 2005, **64:**1190-1194.
82. Jou IM, Shiau AL, Chen SY, Wang CR, Shieh DB, Tsai CS, Wu CL: **Thrombospondin 1 as an effective gene therapeutic strategy in collagen-induced arthritis.** *Arthritis Rheum* 2005, **52:** 339-344.
83. Cronstein BN: **Therapeutic cocktails for rheumatoid arthritis: the mixmaster's guide.** *Arthritis Rheum* 2004, **50:**2041-2043.
84. Schmidt-Weber CB, Rittig M, Buchner E, Hauser I, Schmidt I, Palombo-Kinne E, Emmrich F, Kinne RW: **Apoptotic cell death in activated monocytes following incorporation of clodronate-liposomes.** *J Leukoc Biol* 1996, **60:**230-244.
85. Kinne RW, Schmidt-Weber CB, Hoppe R, Buchner E, Palombo-Kinne E, Nürnberg E, Emmrich F: **Long-term amelioration of rat adjuvant arthritis following systemic elimination of macrophages by clodronate-containing liposomes.** *Arthritis Rheum* 1995, **38:**1777-1790.
86. Barrera P, Blom A, van Lent PL, van Bloois L, Beijnen JH, van Rooijen N, de Waal Malefijt MC, van de Putte LB, Storm G, van den Berg WB: **Synovial macrophage depletion with clodronate-containing liposomes in rheumatoid arthritis.** *Arthritis Rheum* 2000, **43:**1951-1959.
87. Metselaar JM, van den Berg WB, Holthuysen AE, Wauben MH, Storm G, van Lent PL: **Liposomal targeting of glucocorticoids to synovial lining cells strongly increases therapeutic benefit in collagen type II arthritis.** *Ann Rheum Dis* 2004, **63:**348-353.
88. Evans CH, Ghivizzani SC, Lechman ER: **Lessons learned from gene transfer approaches.** *Arthritis Res* 1999, **1:**21-24.
89. Handel ML, Girgis L: **Transcription factors.** *Best Pract Res Clin Rheumatol* 2001, **15:**657-675.
90. Firestein GS: **NF-kappaB: Holy Grail for rheumatoid arthritis?** *Arthritis Rheum* 2004, **50:**2381-2386.
91. Boissier MC, Bessis N: **Therapeutic gene transfer for rheumatoid arthritis.** *Reumatismo* 2004, **56:**51-61.
92. Huber LC, Pap T, Muller-Ladner U, Gay RE, Gay S: **Gene targeting: roadmap to future therapies.** *Curr Rheumatol Rep* 2004, **6:** 323-325.
93. Mueller RB, Skapenko A, Grunke M, Wendler J, Stuhlmuller B, Kalden JR, Schulze-Koops H: **Regulation of myeloid cell function and major histocompatibility complex class II expression by tumor necrosis factor.** *Arthritis Rheum* 2005, **52:**451-460.
94. Brinckerhoff CE, Matrisian LM: **Matrix metalloproteinases: a tail of a frog that became a prince.** *Nat Rev Mol Cell Biol* 2002, **3:** 207-214.
95. Wang X, Liang J, Koike T, Sun H, Ichikawa T, Kitajima S, Morimoto M, Shikama H, Watanabe T, Sasaguri Y, *et al.*: **Overexpression of human matrix metalloproteinase-12 enhances the development of inflammatory arthritis in transgenic rabbits.** *Am J Pathol* 2004, **165:**1375-1383.
96. Heller RA, Schena M, Chai A, Shalon D, Bedilion T, Gilmore J, Woolley DE, Davis RW: **Discovery and analysis of inflammatory disease-related genes using cDNA microarrays.** *Proc Natl Acad Sci U S A* 1997, **94:**2150-2155.
97. O'Hara R, Murphy EP, Whitehead AS, Fitzgerald O, Bresnihan B: **Local expression of the serum amyloid A and formyl peptide receptor-like 1 genes in synovial tissue is associated with matrix metalloproteinase production in patients with inflammatory arthritis.** *Arthritis Rheum* 2004, **50:**1788-1799.
98. Hahn G, Stuhlmuller B, Hain N, Kalden JR, Pfizenmaier K, Burmester GR: **Modulation of monocyte activation in patients with rheumatoid arthritis by leukapheresis therapy.** *J Clin Invest* 1993, **91:**862-870.
99. Hamerlinck FF: **Neopterin: a review.** *Exp Dermatol* 1999, **8:**167-176.
100. Rosengren S, Hoffman H, Bugbee W, Boyle DL: **Expression and regulation of cryopyrin and related proteins in rheumatoid arthritis synovium.** *Ann Rheum Dis* 2005, **64:**708-714.

101. Van Roon JA, Bijlsma JW, van De Winkel JG, Lafeber FP: **Depletion of synovial macrophages in rheumatoid arthritis by an anti-Fc{gamma}RI-Calicheamicin immunoconjugate.** *Ann Rheum Dis* 2005, **64:**865-870.
102. Wijngaarden S, van De Winkel JG, Jacobs KM, Bijlsma JW, Lafeber FP, Van Roon JA: **A shift in the balance of inhibitory and activating Fcgamma receptors on monocytes toward the inhibitory Fcgamma receptor IIb is associated with prevention of monocyte activation in rheumatoid arthritis.** *Arthritis Rheum* 2004, **50:**3878-3887.
103. Liu H, Pope RM: **Phagocytes: mechanisms of inflammation and tissue destruction.** *Rheum Dis Clin North Am* 2004, **30:**19-39.
104. Dao DN, Kremer L, Guerardel Y, Molano A, Jacobs WR Jr., Porcelli SA, Briken V: **Mycobacterium tuberculosis lipomannan induces apoptosis and interleukin-12 production in macrophages.** *Infect Immun* 2004, **72:**2067-2074.
105. Briken V, Porcelli SA, Besra GS, Kremer L: **Mycobacterial lipoarabinomannan and related lipoglycans: from biogenesis to modulation of the immune response.** *Mol Microbiol* 2004, **53:**391-403.
106. Mogensen TH, Paludan SR: **Reading the viral signature by Toll-like receptors and other pattern recognition receptors.** *J Mol Med* 2005, **83:**180-192.
107. Itescu S: **Rheumatic aspects of acquired immunodeficiency syndrome.** *Curr Opin Rheumatol* 1996, **8:**346-353.
108. Cheevers WP, Snekvik KR, Trujillo JD, Kumpula-McWhirter NM, Pretty On Top KJ, Knowles DP: **Prime-boost vaccination with plasmid DNA encoding caprine-arthritis encephalitis lentivirus env and viral SU suppresses challenge virus and development of arthritis.** *Virology* 2003, **306:**116-125.
109. Iguchi T, Kurosaka M, Ziff M: **Electron microscopic study of HLA-DR and monocyte/macrophage staining cells in the rheumatoid synovial membrane.** *Arthritis Rheum* 1986, **29:**600-613.
110. Bresnihan B, Gogarty M, Fitzgerald O, Dayer JM, Burger D: **Apolipoprotein A-I infiltration in rheumatoid arthritis synovial tissue: a control mechanism of cytokine production?** *Arthritis Res Ther* 2004, **6:**R563-R566.
111. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, Zurawski G, Moshrefi M, Qin J, Li X, *et al.*: **IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines.** *Immunity* 2005, **23:**479-490.
112. Chen Q, Carroll HP, Gadina M: **The newest interleukins: recent additions to the ever-growing cytokine family.** *Vitam Horm* 2006, **74:**207-228.
113. Carriere V, Roussel L, Ortega N, Lacorre DA, Americh L, Aguilar L, Bouche G, Girard JP: **IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor *in vivo*.** *Proc Natl Acad Sci U S A* 2007, **104:**282-287.
114. Kim SH, Han SY, Azam T, Yoon DY, Dinarello CA: **Interleukin-32: a cytokine and inducer of TNFalpha.** *Immunity* 2005, **22:**131-142.
115. Brennan F, Beech J: **Update on cytokines in rheumatoid arthritis.** *Curr Opin Rheumatol* 2007, **19:**296-301.
116. Van Roon JA, Glaudemans KA, Bijlsma JW, Lafeber FP: **Interleukin 7 stimulates tumour necrosis factor alpha and Th1 cytokine production in joints of patients with rheumatoid arthritis.** *Ann Rheum Dis* 2003, **62:**113-119.
117. Leonard WJ: **Interleukin-7 deficiency in rheumatoid arthritis.** *Arthritis Res Ther* 2005, **7:**42-43.
118. Toraldo G, Roggia C, Qian WP, Pacifici R, Weitzmann MN: **IL-7 induces bone loss *in vivo* by induction of receptor activator of nuclear factor kappa B ligand and tumor necrosis factor alpha from T cells.** *Proc Natl Acad Sci U S A* 2003, **100:**125-130.
119. Jüngel A, Distler JH, Kurowska-Stolarska M, Seemayer CA, Seibl R, Forster A, Michel BA, Gay RE, Emmrich F, Gay S, *et al.*: **Expression of interleukin-21 receptor, but not interleukin-21, in synovial fibroblasts and synovial macrophages of patients with rheumatoid arthritis.** *Arthritis Rheum* 2004, **50:**1468-1476.
120. Dillon SR, Sprecher C, Hammond A, Bilsborough J, Rosenfeld-Franklin M, Presnell SR, Haugen HS, Maurer M, Harder B, Johnston J, *et al.*: **Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice.** *Nat Immunol* 2004, **5:**752-760.
121. Wong PK, Campbell IK, Egan PJ, Ernst M, Wicks IP: **The role of the interleukin-6 family of cytokines in inflammatory arthritis and bone turnover.** *Arthritis Rheum* 2003, **48:**1177-1189.
122. Romer J, Hasselager E, Norby PL, Steiniche T, Thorn CJ, Kragballe K: **Epidermal overexpression of interleukin-19 and -20 mRNA in psoriatic skin disappears after short-term treatment with cyclosporine a or calcipotriol.** *J Invest Dermatol* 2003, **121:**1306-1311.
123. Liao SC, Cheng YC, Wang YC, Wang CW, Yang SM, Yu CK, Shieh CC, Cheng KC, Lee MF, Chiang SR, *et al.*: **IL-19 induced Th2 cytokines and was up-regulated in asthma patients.** *J Immunol* 2004, **173:**6712-6718.
124. Parrish-Novak J, Xu W, Brender T, Yao L, Jones C, West J, Brandt C, Jelinek L, Madden K, McKernan PA, *et al.*: **Interleukins 19, 20, and 24 signal through two distinct receptor complexes. Differences in receptor-ligand interactions mediate unique biological functions.** *J Biol Chem* 2002, **277:**47517-47523.
125. Wolk K, Kunz S, Asadullah K, Sabat R: **Cutting edge: immune cells as sources and targets of the IL-10 family members?** *J Immunol* 2002, **168:**5397-5402.
126. Wolk K, Kunz S, Witte E, Friedrich M, Asadullah K, Sabat R: **IL-22 increases the innate immunity of tissues.** *Immunity* 2004, **21:**241-254.
127. Caudell EG, Mumm JB, Poindexter N, Ekmekcioglu S, Mhashilkar AM, Yang XH, Retter MW, Hill P, Chada S, Grimm EA: **The protein product of the tumor suppressor gene, melanoma differentiation-associated gene 7, exhibits immunostimulatory activity and is designated IL-24.** *J Immunol* 2002, **168:**6041-6046.
128. Vandenbroeck K, Cunningham S, Goris A, Alloza I, Heggarty S, Graham C, Bell A, Rooney M: **Polymorphisms in the interferon-gamma/interleukin-26 gene region contribute to sex bias in susceptibility to rheumatoid arthritis.** *Arthritis Rheum* 2003, **48:**2773-2778.
129. Radstake TR, Roelofs MF, Jenniskens YM, Oppers-Walgreen B, van Riel PL, Barrera P, Joosten LA, van den Berg WB: **Expression of toll-like receptors 2 and 4 in rheumatoid synovial tissue and regulation by proinflammatory cytokines interleukin-12 and interleukin-18 via interferon-gamma.** *Arthritis Rheum* 2004, **50:**3856-3865.
130. Siren J, Pirhonen J, Julkunen I, Matikainen S: **IFN-{alpha} Regulates TLR-Dependent Gene Expression of IFN-{alpha}, IFN-{beta}, IL-28, and IL-29.** *J Immunol* 2005, **174:**1932-1937.
131. Miossec P, Naviliat M, Dupuy d'Angeac A, Sany J, Banchereau J: **Low levels of interleukin-4 and high levels of transforming growth factor beta in rheumatoid synovitis.** *Arthritis Rheum* 1990, **33:**1180-1187.
132. Allen JB, Wong HL, Costa GL, Bienkowski MJ, Wahl SM: **Suppression of monocyte function and differential regulation of IL-1 and IL-1ra by IL-4 contribute to resolution of experimental arthritis.** *J Immunol* 1993, **151:**4344-4351.
133. Van Roon JA, Lafeber FP, Bijlsma JW: **Synergistic activity of interleukin-4 and interleukin-10 in suppression of inflammation and joint destruction in rheumatoid arthritis.** *Arthritis Rheum* 2001, **44:**3-12.
134. Hermann JA, Hall MA, Maini RN, Feldmann M, Brennan FM: **Important immunoregulatory role of interleukin-11 in the inflammatory process in rheumatoid arthritis.** *Arthritis Rheum* 1998, **41:**1388-1397.
135. Klimiuk PA, Goronzy JJ, Weyand CM: **IL-16 as an anti-inflammatory cytokine in rheumatoid synovitis.** *J Immunol* 1999, **162:**4293-4299.
136. Blaschke S, Schulz H, Schwarz G, Blaschke V, Muller GA, Reuss-Borst M: **Interleukin 16 expression in relation to disease activity in rheumatoid arthritis.** *J Rheumatol* 2001, **28:**12-21.
137. Kaufmann J, Franke S, Kientsch-Engel R, Oelzner P, Hein G, Stein G: **Correlation of circulating interleukin 16 with proinflammatory cytokines in patients with rheumatoid arthritis.** *Rheumatology (Oxford)* 2001, **40:**474-475.
138. Lard LR, Roep BO, Toes RE, Huizinga TW: **Enhanced concentrations of interleukin 16 are associated with joint destruction in patients with rheumatoid arthritis.** *J Rheumatol* 2004, **31:**35-39.
139. Tak PP: **IFN-beta in rheumatoid arthritis.** *Front Biosci* 2004, **9:**3242-3247.
140. Mi Z, Ghivizzani SC, Lechman E, Glorioso JC, Evans CH, Robbins PD: **Adverse effects of adenovirus-mediated gene transfer of human transforming growth factor beta 1 into rabbit knees.** *Arthritis Res Ther* 2003, **5:**R132-R139.