Review

Macrophages in rheumatoid arthritis

Raimund W Kinne, Rolf Bräuer, Bruno Stuhlmüller*, Ernesta Palombo-Kinne and Gerd-R Burmester*

Friedrich Schiller University, Jena, and *Humboldt University of Berlin, Berlin, Germany

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Abstract

The abundance and activation of macrophages in the inflamed synovial membrane/pannus significantly correlates with the severity of rheumatoid arthritis (RA). Although unlikely to be the 'initiators' of RA (if not as antigen-presenting cells in early disease), macrophages possess widespread pro-inflammatory, destructive, and remodeling capabilities that can critically contribute to acute and chronic disease. Also, activation of the monocytic lineage is not locally restricted, but extends to systemic parts of the mononuclear phagocyte system. Thus, selective counteraction of macrophage activation remains as efficacious approach to diminish local and systemic inflammation, as well as to prevent irreversible joint damage.

Keywords: cytokine, fibroblast, macrophage, monocyte, nitric oxide, peripheral blood, reactive oxygen species, rheumatoid arthritis, synovial membrane, T-cell

Introduction

Macrophages appear to play a pivotal role in RA because they are numerous in the inflamed synovial membrane and at the cartilage–pannus junction. They show clear signs of activation, such as overexpression of major histocompatibility complex class II molecules, proinflammatory or regulatory cytokines and growth factors [eg IL-1, IL-6, IL-10, IL-13, IL-15, IL-18, tumour necrosis factor (TNF)-α and granulocyte–macrophage colony-stimulating factor (GM-CSF)], chemokines and chemoattractants [eg IL-8, macrophage inflammatory protein (MIP)-1 and monocyte chemoattractant protein (MCP)-1], metalloproteinases and neopterin [1–4]. It is unlikely that macrophages occupy a causal position in the pathogenesis of RA (except for their function as antigen-pre-

senting cells in the hypothesis of a primary autoimmune disorder) [5]. However, these cells of the innate immune system possess broad proinflammatory, destructive and remodelling capacities, and considerably contribute to inflammation and joint destruction both in the acute and chronic phases of RA. Also, activation of the monocytic lineage is not restricted to synovial macrophages, but extends to circulating monocytes and other cells of the mononuclear phagocyte system, including precursors of the myelomonocytic lineage in the bone marrow [2,3,6].

Thus, even before the cause of RA is identified [whether it is an infectious cause, or a clearly defined (auto)antigen or a genetic alteration (or several)], several facts render

monocytes/macrophages attractive therapeutic targets. The first is the correlation between radiological progression of joint destruction and degree of synovial macrophage infiltration [7]. Second, the coincidence of therapeutic efficacy of conventional antirheumatic therapy with downregulation of functions of the mononuclear phagocyte system [8,9] is in accord with the increasing knowledge of macrophage-specific effects of such drugs [10]. Third, the efficacy of biological therapies directed at cytokines produced predominantly by macrophages has been demonstrated [11,12]. Fourth, conventional or experimental drugs can be targeted at macrophages, including their subcellular compartments [2]. The final factor in support of targeting monocytes/macrophages for treatment of RA is the differential activation of intracellular signal transduction pathways that underlie different macrophage effector functions, in conjunction with the availability of more specific inhibitors of key metabolic enzymes and/or particular signal transduction pathways [13–15].

Differentiation of the mononuclear phagocyte system in rheumatoid arthritis

Cells of the myelomonocytic lineage differentiate into several cell types that are involved in disease (ie monocytes/macrophages, osteoclasts and dendritic cells). As a result of their marked plasticity, these differentiation pathways can be influenced by particular pathophysiological stimuli (eg an excess/imbalance of cytokines or growth factors), resulting in altered differentiation or maturation if regulatory mechanisms fail. In RA, such alterations have been already described at several levels (ie in inflamed joints, peripheral blood and bone marrow).

In the RA synovial membrane, a first differentiation step is that from recently immigrated monocytes to mature macrophages [16]. These subsets differentially colonise the synovial sublining and lining layer, respectively, as well as the superficial and deep layers of the lining [7]. A possible functional diversity in these areas, which is emphasized by the expression of different activation markers and adhesion molecules [17], may differentially contribute to disease progression [7].

Locally, synovial macrophages also differentiate into stimulatory or inhibitory subpopulations, which are known to influence T-cell reactivity differentially [18]. In experimental arthritis, for example, immunoregulatory macrophages greatly affect T-cell responses to arthritogenic proteins [19] and, upon nonselective targeting, influence clinical and histopathological improvement in arthritis [20]. In RA, macrophage subpopulations may be responsible for the separate synthesis of proinflammatory (eg IL-1 and TNF-α) or regulatory cytokines (eg IL-10), the balance of which is critical to perpetuation of disease [21,22]. A subset of synovial macrophages may also exert a predominant role in angiogenetic processes [23].

In the RA synovial fluid, a subset of mononuclear cells presents with a double phenotype of activated T cells and macrophage-like cells [24]. Whether this promiscuity is related to plasticity of common precursors, or is the epiphenomenon of a central differentiation defect remains to be clarified.

Alterations in the macrophage lineage are also evident in extrasynovial compartments. In the bone marrow, RA patients with active or severe disease display faster generation of CD14^{+} myelomonocytic cells and faster differentiation into human leucocyte antigen (HLA)-DR^{+} cells than do control individuals [25]. Myeloid precursors are also elevated in the bone marrow adjacent to rheumatoid joints, in correlation with the local levels of IL-1 [26]. A common potential trigger for these bone marrow alterations may be the altered monokine (eg IL-1, IL-6, TNF-α) or growth factor milieu (eg GM-CSF), which builds up in the circulation [6,8], and in the bone marrow [27] of RA patients. On the other hand, the *in vitro* differentiation of bone marrow precursor cells becomes insensitive to GM-CSF [25], suggesting intrinsic alterations in the myelomonocytic lineage.

Possible bone marrow anomalies may also underlie the presence of highly proliferative potential colony-forming cells in the peripheral blood of RA patients with severe disease and high incidence of interstitial pulmonary involvement [28,29], a factor of poor prognosis in RA. Finally, bone marrow stromal cells also overexpress bone marrow stromal antigen (BST)-1, a pre-B-cell growth factor that is significantly elevated in the sera of patients with severe RA [30], with growth inhibition effects on monocytes/macrophages. These observations, as well as that of the existence of a macrophage activation syndrome in severe cases of systemic juvenile RA [31], suggest that the spectrum of arthritis severity may be associated with the degree of systemic activation of monocytes/ macrophages. This is also supported by the extra-articular terminal differentiation of macrophages within rheumatoid nodules, the latter a sign of clinical severity [32,33].

The involvement of the myelopoietic system in RA may also partly explain the mode of action of slow-acting antirheumatic drugs, possibly targeting altered precursors [34], or that of stem-cell transplantation therapy [35].

Activation of the mononuclear phagocyte system in rheumatoid arthritis
Synovial compartments
Synovial membrane
In the RA synovial membrane, a surface layer of HLA-DR^{+}, CD14^{+} and CD68^{+} macrophages is typically followed by a layer of fibroblasts [2]. Below the lining layer, macrophages are distributed in lymphoid aggregates or in diffuse infiltrates, in the former case adjacent to activated CD4^{+} lymphoid cells and in the latter case near CD8^{+}

T cells [36], suggesting active participation in possible (auto)immune processes. In addition, macrophages are located close to synovial fibroblast-like cells that display atypical morphology, which are believed to be centrally involved in tissue destruction.

The degree of macrophage infiltration/activation correlates not only with the joint pain and general inflammatory status of the patient [37], but also with the radiological progression of permanent joint damage [7], the disease feature that ultimately determines quality of life.

In chronic RA, the prevalence of certain histological configurations may represent an important variable for the clinical course. High TNF-α and IL-1β production, for example, may be associated with granulomatous synovitis, a rare condition that is more frequently associated with subcutaneous rheumatoid nodules [32]. Conversely, these cytokines appear to be modestly elevated in diffuse synovitis, which may be associated with seronegative RA [32]. These features may also explain some variability among studies on the abundance of TNF-α and/or TNF-α receptor expression in the RA synovial membrane [38,39], and, possibly, the variable sensitivity to anti-TNF-α therapy [11].

Myeloid-related dendritic cells are also enriched in RA synovial compartments. Their efficacy as antigen-presenting cells and their interdigitating location in perivascular lymphoid aggregates are optimal prerequisites for the presentation of putative arthritogenic antigens to T cells and for the regulation of B cells [40].

Cartilage–pannus and bone–pannus junction
At the site of tissue destruction, macrophages express significant amounts of the inflammatory cytokines IL-1, TNF-α and GM-CSF [2] and contribute to the production of the proteases collagenase, stromelysin, gelatinase B and leucocyte elastase [41]. Although gelatinase B levels positively correlate with disease progression and severity [42], the potential of macrophages to degrade cartilage matrix components directly may be modest [41], assigning macrophages the position of amplifyers of the pathogenetic cascade (especially via activation of fibroblasts) rather than primary effectors of tissue destruction. The situation may be quite different at the bone–pannus junction, where osteoclasts derived from the myelomonocytic lineage strongly contribute to bone erosion [43], possibly under the influence of local cell–cell contact and abundant cytokines.

Peripheral blood
The activation of circulating monocytes in RA, although unclear in its extent [44], is evidenced by the following: spontaneous production of prostanoids and prostaglandin E_2 [45], cytokines [8,46,47], soluble CD14 [2] and neopterin [8], the latter a molecule exclusively produced by human mononuclear phagocytes in correlation with disease activity [48]; increased production of the matrix-degrading enzyme gelatinase B [42,49] and the metalloprotease inhibitor tissue inhibitor of metalloproteinase (TIMP)-1 [50]; expression of manganese superoxide dismutase, a critical enzyme for the control of oxygen radicals [50]; increased phagocytic activity [51]; increased integrin expression and monocyte adhesiveness [47,52]; presence of activated suppressor monocytes [18,53]; and, more generally, gene activation with a pattern closely resembling the synovial activation pattern.

Differential analysis of gene patterns in RA monocytes collected upon initial and final therapeutic leukapheresis [6] (a procedure that induces clinical remission in severe RA, presumably by reducing the degree of monocyte activation) [8,54] has recently shed light on gene expression at different stages of monocyte activation. In addition to the expected cytokines (IL-1α, IL-1β, IL-6, TNF-α), gene activation in the florid stage of disease has also been documented for growth-related oncogene protein (GRO)-α/ melanoma growth-stimulatory activity, MIP-2/GRO-β, ferritin, α_1-antitrypsin, lysozyme, transaldolase, Epstein-Barr virus encoded small nuclear RNA (EBER)-1/EBER-2-associated protein, thrombospondin-1, an angiotensin receptor-II carboxyl-terminal homologue, the RNA polymerase-II elongation factor, and for five unknown/functionally undefined genes [6]. Because a number of these molecules (IL-1α, IL-1β, IL-6, TNF-α, GRO-α, ferritin, lysozyme and thrombospondin-1) are also (over)expressed in RA joints [6], monocytes appear to be preshaped in a 'rheumatoid' phenotype before their entry into the inflamed synovial tissue. This is further supported by overexpression of the human cartilage glycoprotein gp39 [55], a late macrophage differentiation marker [56], in circulating monocytes and tissue macrophages. The findings regarding novel or functionally undefined genes also indicate that the extent of systemic monocyte/macrophage activation remains to be fully explored.

Stimulation/regulation of monocyte/ macrophage activation in rheumatoid arthritis
Cell–cell interaction
A significant part of macrophage effector responses occurs in the absence of soluble stimuli, through cell contact-dependent signalling with several inflammatory cells [57,58].

T cell–macrophage interaction
Accessory, inflammatory, effector and inhibitory macrophage functions can be stimulated by paraformaldehyde-fixed T cells or plasma membranes of T cells [57], provided that these are preactivated and express the surface molecules that are involved in such stimulation (eg CD69) [57]. In response to such interaction, monocytes produce metalloproteinases, IL-1α and IL-1β [59,60]. Also, T cells that are prestimulated in an antigen-mimicking

manner stimulate TNF-α and IL-10 production, once they are in contact with monocytes [61]. Conversely, fixed T cells stimulated in an antigen-independent manner (ie with IL-15, IL-2, or a combination of IL-6 and TNF-α) induce monocyte production of TNF-α, but not IL-10 [62]. These findings have resulted in the hypothesis that early RA reflects antigen-specific T cell–macrophage interactions. Conversely, chronic RA may be associated with antigen-independent interactions, which are dominated by an exuberant cytokine milieu. This model would also explain the relative paucity of IL-10 in the RA synovial membrane (see below).

Fibroblast–macrophage interaction
Because macrophages and fibroblasts appear to be the most active cells in the RA synovium, their interaction is particularly interesting in view of the resulting inflammation and tissue damage. Indeed, the mere contact of these cells elicits the production of IL-6, GM-CSF and IL-8 [63]. The cytokine output can be enhanced or downregulated not only by addition of proinflammatory or regulatory cytokines (eg IL-4, IL-10, IL-13 or IL-1 receptor antagonist), but also by neutralization of the CD14 molecule [63]. Also, *in vitro* model systems show significant cartilage degradation by cocultures of mouse fibroblasts and macrophages, a response markedly exceeding that observed with each culture alone [64]. More recent studies [65] have shown that purified human synovial fibroblasts cocultured with myelomonocytic cell lines induce cartilage degradation *in vitro*. The mechanism of this response, however, seems to be paracrine- and not cell contact-mediated, because cartilage degradation is most effectively blocked by a mixture of anti-IL-1 and anti-TNF-α monoclonal antibodies.

Soluble stimuli
Proinflammatory cytokines
IL-15, an IL-2-like cytokine with chemoattractant properties for memory T cells, is highly augmented in RA synovial fluid, being produced by lining layer cells, including macrophages [66]. Notably, IL-15-stimulated peripheral blood or synovial T cells induce macrophages to produce IL-1β, TNF-α, IL-8 and MCP-1 [62,67,68], but not IL-10 [61]. Because IL-15 is also produced by macrophages themselves, this cytokine may (re)stimulate T cells, possibly contributing to a self-perpetuating proinflammatory loop [67].

The lymphokine IL-17, which is produced in approximately 90% of RA synovial explant cultures, but only in 16% of osteoarthritis cultures, strongly stimulates macrophages to produce IL-1 and TNF-α [69]. The induction of TNF-α production can be completely reversed by addition of IL-10 [69]. IL-17, which is present in T cell-rich areas of RA synovial samples, is exclusively produced by T-helper (Th)0 or Th1 clones that are derived from the synovial membrane

or synovial fluid of RA patients [70]. In addition, IL-17 indirectly induces the formation of osteoclasts from progenitor cells [71] and enhances the production of nitric oxide (NO) in articular chondrocytes [72], thus potentially contributing to cartilage and bone destruction.

In the RA synovial membrane IL-18, a cytokine of the IL-1 family [73], is expressed most prominently in CD68+ macrophages that are contained in lymphoid aggregates [4]. CD14+ macrophages of the RA synovial fluid also express the IL-18 receptor [4]. IL-18, either alone or in concert with IL-12 and IL-15, strongly enhances the production of IFN-γ, TNF-α, GM-CSF and NO by cultured synovial cells. Treatment with recombinant murine IL-18 markedly aggravates experimental arthritis [4], indicating that IL-18 has proinflammatory effects in this disorder.

Bacterial/viral components
The ability of bacterial toxins or superantigens to initiate proinflammatory responses that are characterized by secretion of macrophage-derived cytokines is relevant in view of the possible micro-organism aetiology of RA. Lipopolysaccharide, for example, binds to macrophages through the CD14 receptor and, *in vitro*, stimulates the production of IL-1β, TNF-α and MIP-1α [74]. Staphylococcal enterotoxin B, also a potent macrophage-activating factor *in vitro* and *in vivo*, enhances arthritis in MRL-*lpr/lpr* mice. In this case, anti-TNF-α therapy reverses both the severe wasting effects of staphylococcal enterotoxin B and the incidence of arthritis, indicating that TNF-α is central in this system [75]. Lipoarabinomannan, a mycobacterial lipoglycan that is involved in attenuating host immune responses and entry of mycobacteria into macrophages [76], induces monocyte chemotaxis, as well as selective production of IL-12 and subsequent differentiation of T cells toward a Th1-like phenotype [77,78]. It also stimulates macrophages to produce TNF-α, GM-CSF, IL-1, IL-6, IL-8 and IL-10 [76].

More generally, the prolonged persistence of obligate or facultative intracellular pathogens in macrophages may directly lead to the development of arthritis. This is the case with the Ross River virus, which causes human epidemic polyarthritis [79], or with the caprine-arthritis encephalitis lentivirus, which causes a disorder in which viral replication within macrophages correlates with the severity of tissue pathology [80]. Further interesting cases are arthritis associated with human immunodeficiency virus-1 infection, which, as with all other manifestations of this disease, is due to virus tropism for macrophages [81], and that associated with human parvovirus B19 [82].

Hormones
RA undergoes clinical fluctuations during the menstrual cycle and during pregnancy. These observations have a pathophysiological basis in the expression of sex hormone

receptors on RA synovial macrophages [83]. Indeed, physiological concentrations of oestrogens stimulate the production of the proinflammatory cytokine IL-1 by RA macrophages. Conversely, higher oestrogen concentrations inhibit IL-1 production, perhaps mimicking the clinical improvement that occurs during pregnancy [83]. Thyroid or other neuroendocrine hormones can also influence RA, at least partly through actions on macrophages [84,85].

Regulation of monocyte/macrophage activation in rheumatoid arthritis
Regulatory cytokines
The anti-inflammatory cytokine IL-4 is believed to play a protective role in arthritis, although its virtual absence from synovial samples points to the lack of protective mechanisms, rather than to active regulation [86]. This Th2-like cytokine downregulates monocyte/macrophage cytotoxicity and cytokine production [87], including that of TNF-α and TNF-α receptors [88], as well as IL-15-induced chemokine production [68]. Notably, IL-4 decreases IL-1β production while increasing IL-1 receptor antgonist production, thus suggesting a 'coordinated' anti-inflammatory approach [89]. IL-4 also decreases the mRNA production of cyclo-oxygenase 2 and cytosolic phospholipase A_2, thereby reducing the levels of prostaglandin E_2 [90,91]. In RA, IL-4 decreases monokine production in *ex vivo* synovial specimens [86], or TNF-α receptor expression by synovial fluid macrophages [88]. Importantly, IL-4 reduces bone resorption [86] as well as synovial proliferation *in vitro* [22]. Consistently, experimental therapy with IL-4 clearly suppresses streptococcal cell wall-induced arthritis, a strongly macrophage-dependent model [89]. In considering a combined antimacrophage and immunotherapeutic approach in autoimmunity, IL-4 should be the elective molecule because of its ability to regulate macrophages and to shift the cytokine pattern from a Th1-like to a Th2-like predominance [92]; however, induction of fibrosis may limit its therapeutic applicability.

IL-10 is a macrophage-derived cytokine with clear autocrine functions [93]. Accordingly, IL-10 reduces HLA-DR expression and antigen presentation in monocytes [87] and inhibits the production of proinflammatory cytokines, GM-CSF and Fcγ receptors by synovial macrophages [87]. Consistently with cytokine and chemokine downregulation, IL-10 clearly suppresses experimental arthritis [94].

In spite of IL-10 elevation in serum and synovial compartments of RA patients [61,87], some studies [95] have suggested a relative deficiency in IL-10. A combined IL-4/IL-10 deficiency may therefore shift the cytokine balance to a proinflammatory predominance. Because of the autocrine effects of IL-10 [93], this may further enhance the 'rheumatoid' macrophage imprint. Although recombinant IL-10 appears to be an optimal candidate for treatment of RA [96], the induction of soluble and membrane TNF-α receptors on monocytes and synovial fluid macrophages [88], as well as the lack of efficacy in some cohorts of RA patients [97], currently question the broad applicability of this treatment.

IL-11 is present in synovial membrane, synovial fluid and sera of RA patients, and blockade of endogenous IL-11 increases endogenous TNF-α production in RA synovium *in vitro* [98], whereas recombinant human IL-11 reduces the production of TNF-α and IL-12 from activated macrophages [99]. IL-11 may represent another example of a paracrine regulatory loop, because IL-1α and TNF-α synergistically stimulate the production of IL-11 in rheumatoid synovial fibroblasts [100]. Treatment with IL-11 decreases clinical severity and prevents joint damage in murine collagen-induced arthritis [101], which is in accord with high levels of IL-11 mRNA in clinically uninvolved paws [102]. Clinical studies with recombinant human IL-11 have been initiated in several inflammatory conditions, including RA [103].

Similarly to IL-4 and IL-10, IL-13 exerts suppressive effects in experimental arthritis, probably through a selective effect on monocytes/macrophages [104]. In RA, IL-13 is produced by synovial fluid mononuclear cells, which, when exposed to exogenous IL-13, diminish their own production of IL-1 and TNF-α [105].

The question regarding whether IL-16 is proinflammatory or anti-inflammatory is still under debate [106]. In the RA synovial membrane, IL-16 is present in CD68$^-$ synovial fibroblasts [107] and CD8$^+$ T cells [108]. In a human synovium/severe combined immunodeficiency mouse chimera, IL-16 behaves as an anti-inflammatory cytokine by strongly reducing levels of mRNA for IFN-γ, IL-1β and TNF-α [but not for transforming growth factor (TGF)-β], and by decreasing the numbers of cells that express IFN-γ and TNF-α in the human implant [108]. IL-16 therefore appears to be yet another potential candidate for RA treatment, but one that requires care in view of its strong T-cell chemoattractant features.

Monocyte/macrophage effector molecules in rheumatoid arthritis
Proinflammatory cytokines
Tumour necrosis factor-α
TNF-α is a pleiotropic cytokine that increases the expression of cytokines, adhesion molecules, prostaglandin E_2, collagenase and collagen by synovial cells [109]. In RA, TNF-α is mostly produced by macrophages in the synovial membrane and at the cartilage–pannus junction [38] and is believed to be a proximal cytokine in the inflammatory cascade. While, on average, approximately 5% of synovial cells express TNF-α mRNA *in situ* [110], the degree of TNF-α expression in the synovial tissue appears to depend on the prevailing histological configuration [32].

The importance of TNF-α is evidenced by several experimental and clinical observations: lymph node TNF-α production precedes clinical synovitis in experimental arthritides [111,112]; neutralization of TNF-α suppresses collagen-induced arthritis and reduces inflammation in human/murine severe combined immunodeficiency arthritis [38,113]; TNF-α, in combination with IL-1, is a potent inducer of synovitis [114]; transgenic, deregulated expression of TNF-α causes development of chronic arthritis [115]; the TNF-α levels in the synovial fluid correlate with the number of lining macrophages and with the degree of radiologically assessed bone erosion [116]; and serum TNF-α and soluble TNF receptors 1 and 2 are increased in active, systemic juvenile rheumatoid arthritis, including some cases of macrophage activation syndrome [31].

TNF-α exists as a membrane-bound and a soluble form. Transmembrane TNF-α appears to be involved in local, cell contact-mediated processes, and appears the prime stimulator of the R75 receptor [117]. Interestingly, the transgenic expression of this form alone is sufficient to induce chronic arthritis [118]; likewise, a mutant membrane TNF-α, which utilizes both R55 and R75 receptors, can also cause arthritis [119]. Conversely, the soluble form of TNF-α, shed via metalloprotease cleavage from the membrane-bound form [120], primarily stimulates the R55 receptor, acting transiently and at a distance [117].

Tumour necrosis factor-α receptors
TNF-α receptors are found in synovial tissue and fluid of RA patients [38,39,121], especially in severe disease [121]. There are two known TNF-α receptors: R55 (TNF-α receptor 1; high-affinity receptor) and R75 (TNF-α receptor 2; low-affinity receptor). The resulting stable R55 or transient R75 character of the ligand–receptor complex mediates different cellular responses to soluble and transmembrane TNF-α [122]. In general, TNF-α receptors can operate independently of one another, cooperatively, or by 'passing' TNF-α to one another [117]. This complexity may explain the tremendous sensitivity of target cells to minute concentrations of TNF-α, as well as the considerable variety of its effects. TNF-α receptors can also be shed, binding to soluble TNF-α and hence acting as natural inhibitors in disease [123].

Consistent with the pivotal role of TNF-α in arthritis [124], clinical trials with intravenous administration of chimaeric anti-TNF-α monoclonal antibodies or TNF-α receptor constructs have shown remarkable efficacy in acute disease [11], with long-term safety [125] and slowing-down of radiological disease progression [126,127].

Also, in accordance with the activating role of TNF-α in leucocyte extravasation [128], anti-TNF-α treatment reduces endothelial E-selectin and vascular cell adhesion molecule-1 in synovial samples, as well as the levels of E-selectin, intercellular adhesion molecule-1, IL-8 and vascular endothelial growth factor in the circulation [129]. This downregulartion may lead to deactivation of the vascular endothelium and suppression of angiogenesis, as substantiated in animal studies [129].

Interleukin 1
In RA, IL-1 gene expression is found predominantly in CD14+ macrophages [130], and IL-1 levels in the synovial fluid significantly correlate with joint inflammatory activity [21]. This cytokine, which is believed to act in sequence after TNF-α [38], appears to mediate most of the articular damage in arthritis [21], because it induces proteoglycan degradation and inhibition of proteoglycan synthesis [131]. Also, IL-1 induces the production of the metalloproteases stromelysin and collagenase [21], and enhances bone resorption [132]. In RA, the balance between IL-1 and its physiological inhibitor IL-1 receptor antagonist is shifted in favour of IL-1, indicating a dysregulation that may be crucial in promoting chronicity.

Chemokines
Several studies have documented the existence of a positive feedback between the macrophage-derived TNF-α and IL-1 and chemotactic factors for monocytes, IL-8 and MCP-1 [133]. These factors are produced by synovial macrophages in an autocrine manner [134]. The highest levels of secreted IL-8 are observed in patients with seropositive RA, indicating a correlation with a particularly vigorous macrophage activation [135]. Significantly, IL-8 derived from synovial macrophages is a powerful promoter of angiogenesis, thus providing a link between macrophage activation and the prominent neovascularization of the RA synovium [136].

Anti-inflammatory/regulatory cytokines
Macrophages also generate anti-inflammatory cytokines, most notably IL-1 receptor antagonist and IL-10 (the latter described under Regulatory cytokines, above), both cytokines being engaged in autocrine regulatory loops.

Differentiated macrophages constitutively express IL-1 receptor antagonist, which binds to IL-1 receptors without evoking physiological responses [21]. Significantly, this protein is upregulated by proinflammatory cytokines, including IL-1 itself or GM-CSF, inducing strong anti-inflammatory effects [21]. By means of this feedback mechanism, macrophages can therefore contribute to the termination of inflammatory reactions. The critical relevance of IL-1 in chronic RA [21], as well as the imbalance between IL-1 and IL-1 receptor antagonist, constitute the rationale for apparently successful therapy with IL-1 receptor antagonist [12,21,137].

Cytokines with a dual role in arthritis
Interleukin-6
IL-6 is the most strikingly elevated cytokine in RA, especially in the synovial fluid during acute disease [138]. The

acute rise is consistent with the role of IL-6 in acute-phase responses. However, although 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 [139], this cytokine may also protect cartilage in acute disease but promote excessive bone formation in chronic disease [140]. Although IL-6 is mostly produced by synovial fibroblasts and only partly by macrophages [141], two findings suggest that the striking IL-6 rise is a prominent outcome of macrophage activation: the morphological vicinity of IL-6-expressing fibroblasts with CD14+ macrophages in the RA synovial tissue [141]; and the *in vitro* and coculture studies [63] that showed that IL-1 stimulates IL-6 production.

Transforming growth factor-β
In RA, different forms of TGF-β and TGF-β receptors are expressed by macrophages in the lining and sublining [142], as well as at the cartilage–pannus junction [143] and in the synovial fluid [144]. TGF-β is a main regulator of connective tissue remodelling, controlling both matrix production and degradation [145]. In experimental animals, TGF-β can induce synovial inflammation, but can also suppress acute and chronic arthritis [144]. In the latter case, TGF-β can act as a regulatory molecule, especially by counteracting some of the effects of IL-1, including metalloprotease production [145] and phagocytosis of collagen [146]. On the other hand, synovial fluid TGF-β induces the expression of the FcγRIII macrophage receptor, the stimulation of which induces release of tissue-damaging reactive oxygen species [147]. The potential relevance of FcγRs for the pathogenesis of arthritis has recently been re-emphasized [148,149]. Likewise, TGF-β is a potent chemotactic factor for leucocytes, promoting monocyte adhesion and possibly resulting in excessive inflammatory infiltration in chronic disease [150]. Another limit to the therapeutic potential of TGF-β is the enhancement of synovial fibroblast proliferation [144].

The inhibiting effects of TGF-β on metalloproteases are also controversial. While inhibition of gelatinase B and collagenase suggest a protective role in tissue destruction [151], the increase collagenase-3 in chondrocytes may aggravate cartilage damage [152]. The effects of TGF-β on TIMP are also ambiguous [153], because the regulation of matrix metalloproteinase and TIMP may depend on different tissue domains (superficial versus deep cartilage layers) and may also vary for intracellular and extracellular digestion of collagen [146,152]. Metalloproteases themselves can also affect TGF-β, by regulating the shedding of latent TGF-β that is attached to decorin [154], and thereby creating a loop that may enhance disease.

Nitric oxide and reactive oxygen species
In RA, variable numbers of synovial lining macrophages may represent a source of NO [155,156]. Synovial cells exposed to NO increase their TNF-α production [156], possibly adding to the mechanisms that promote synovitis [157]. NO may be relevant to arthritis also because of its effects on bone remodelling [158]. However, NO can also exert protective effects in experimental autoimmunity [159], limiting perhaps the antiarthritic impact of selective antagonists for human inducible NO synthase [160].

RA macrophages also produce reactive oxygen species [161], which are involved in distal inflammatory processes. Enhanced mitochondrial production of reactive oxygen species in RA blood monocytes correlates with plasma levels of TNF-α, confirming the stimulatory effect of this cytokine on the production of reactive oxygen species [161].

In general, the link between macrophage activation and molecules with dual activity (be they macrophage products or external stimuli, the latter being soluble, membrane-bound, stimulatory, or suppressive factors) and the number of different receptors that are involved in such stimulation may result not only in potent proinflammatory functions of macrophages, but also in powerful anti-inflammatory and tissue repair functions. Finally, macrophage stimuli and macrophage responses rely heavily on autocrine regulatory mechanisms, which makes it difficult to discern the cause from the effect.

Lessons from experimental models of arthritis
Experimental arthritides with histological similarities to human RA [2] have confirmed the role(s) of macrophages and/or their products in synovitis [2], including the following: macrophage infiltration and activation patterns; migration mechanisms of bone marrow-derived monocytes; maturation into effector and immunoregulatory subpopulations; activation of circulating monocytes and extra-articular macrophages; leading role of TNF-α; sensitivity to cytokine-based therapy, including gene therapy approaches; and sensitivity to reduction of monocyte recruitment or depletion of activated macrophages/osteoclasts.

In addition to the TNF-α transgenic models of arthritis, two recent transgenic manipulations are worth mentioning. The first, a back-cross of TNF-α transgenic mice with arthritis-susceptible DBA/1 mice [162], results in arthritis with enhanced production of TNF-α, IL-1 and IL-6 in the synovial membrane, but with remarkable paucity of macrophage and lymphocyte infiltration. The second, a back-cross of a T-cell receptor transgene with the non-obese diabetes (NOD) strain (K/B×N) [163], is characterized by erosive arthritis with predominant macrophage infiltration and other striking similarities to human RA: synovitis of the distal joints, lymphocyte clustering close to macrophage infiltrates and predominant polymorphonuclear leucocyte exudation in the synovial fluid. Strikingly, the autoantigen recognized by both T cells and B cells in this model has recently been identified as the ubiquitous

glycolytic enzyme glucose-6-phosphate isomerase, lending strong support to the concept that general breakage of tolerance can lead to aggressive arthritis [164].

Treatment of human rheumatoid arthritis with conventional antimacrophage approaches

The definition of the role of macrophage-derived cytokines in the perpetuation of RA [2], of the pathophysiological and therapeutic dichotomy between inflammation and cartilage destruction, and of the crucial significance of activated macrophages in the synovial membrane in relationship to permanent joint damage [7] have prompted a radical re-evaluation of the current regimens of anti-inflammatory and disease-modifying treatments. In addition, research concerning conventional agents with antimacrophage effects is now aimed at potentiating such effects.

Disease-modifying antirheumatic therapy
Empirically introduced disease-modifying antirheumatic drugs (DMARDs) possess a whole array of anti-macrophage effects [10].

Gold compounds
Administration of gold compounds to RA patients results in gold accumulation in the lysosomes of synovial macrophages, especially in lysosome-rich sublining macrophages [165]. In monocytes, gold compounds inhibit Fc and C3 receptor expression, oxygen radical generation and IL-1 production [2]. Through their effects on macrophages as accessory cells, gold compounds also inhibit T-cell proliferation in response to antigen or mitogen [2]. Gold compounds inhibit the production of IL-1, IL-8 and MCP-1 [166] and decrease monocyte chemotaxis *in vitro* [2]. In the synovial lining, this is paralleled by a significant decrease in macrophage numbers and IL-1, IL-6 and TNF-α production [9,10]. *In vitro*, gold salts also inhibit angiogenic properties of macrophages, probably through their thiol moiety [167], which colocalizes in macrophage lysosomes together with gold [165]. In experimental arthritis, gold salts seem much more effective as long-term disease-modifying drugs than as anti-inflammatory agents [168].

Methotrexate
One of the most effective DMARDs, methotrexate also impairs chemotaxis of blood monocytes [2] and monokine production [169], while increasing the production of cytokine inhibitors, including soluble TNF-α receptor R75. Because methotrexate shifts the IL-1/IL-1 receptor antagonist balance in favour of IL-1 receptor antagonist, this drug may pharmacologically correct the imbalance between these two mediators [169]. A change in the monokine balance, including that of TNF-α [169], may indirectly cause a selective decrease in collagenase production in the synovial tissue. A surprising caveat of methotrexate therapy, however, is the accelerated formation of rheumatoid nodules in some patients [170].

Antimalarials
Antimalarials are endowed with significant antirheumatic effects in early RA [171], but are apparently less effective than gold. They are are attractive because of their limited toxicity. Their tropism for lysosomes [172] is probably the cause for their slow accumulation in macrophages, in which they inhibit the release of arachidonate and the production of prostaglandin E_2 via phosholipase A_2 inhibition [173]. At high concentrations, antimalarials also inhibit the production of IL-1 and TNF-α in lipopolysaccharide-stimulated macrophages [174].

Corticosteroids
The potent anti-inflammatory effects of corticosteroids in RA can be at least partly explained by transcriptional downregulation of the inflammatory cytokines IL-1 and IL-6, or, as recently reported, transcriptional and post-transcriptional downregulation of TNF-α in monocytes [175]. Corticosteroids may also affect the balance of the functionally distinct membrane-bound and soluble TNF-α [117]. Interestingly, *in vitro* studies suggest that addition of low doses of IL-4 and IL-10 decreases the dose of corticosteroids that is necessary to downregulate TNF-α, possibly via a coordinated attack on activated macrophages. Corticosteroid also decrease the production of IL-8 and MCP-1 [134,166]; this, once optimally exploited, may limit the self-perpetuating ingress of monocytes into the inflamed joint.

Nonsteroidal anti-inflammatory drugs
Aspirin reduces the production of prostaglandin E_2 through acetylation of the isoforms 1 and 2 of the cyclo-oxygenase. Although its use is limited by the gastric side effects (mostly dependent on cyclo-oxygenase-1 inhibition), there is a renewed interest in aspirin derivatives that potently and selectively inactivate the inducible cyclo-oxygenase-2 isoform in isolated macrophages and in local inflammation [15,176]. Cyclo-oxygenase-2-dependent mechanisms selectively induce the production of IL-6 [177], the cytokine that is most highly increased in the RA synovial fluid. Aspirin may affect macrophages also by decreasing the TNF-α production via nuclear factor-κB (NF-κB) mechanisms [178].

Experimental antimacrophage therapy
Counteraction of monocyte/macrophage activation at a cellular level
Leukapheresis
In RA, repeated leukapheresis has proven effective in depleting the bloodstream of activated monocytes [8,54], leading to reduced expression of differentiation markers, DR antigens, cytokines, neopterin and prostaglandin E_2 [6,8,54].

Apoptosis-inducing agents
A theoretical way of counteracting activated macrophages or osteoclasts is to eliminate them physically from the

inflammatory foci, or from systemic loci that are relevant to disease. Incorporation of liposome-encapsulated bisphosphonates by activated macrophages, for example, induces apoptotic cell death in these cells [179], a process that circumvents secondary tissue damage by restraining cellular organelles in apoptotic vesicles. Systemic application to arthritic rats counteracts not only joint swelling, but also joint destruction and subchondral bone damage [2,180]. The DMARD bucillamine also appears to display apoptotic effects in monocyte lines [181]. Notably, liposome encapsulation can also be exploited for selective delivery of macrophage-modulating drugs [2] and for gene therapy constructs [182].

Control of gene transcription
The transcription of most cytokine genes in monocytes/macrophages depends on the activation of NF-κB and nuclear factor-κM transcription factors [183] or that of the activator protein (AP)-1 complex. In RA synovial macrophages, the expression of NF-κB is much more pronounced than that of AP-1 [184], a selectivity that may bear important therapeutic implications [185]. Accordingly, the antiarthrtic effects of IL-4 may be based on the selective suppression of NF-κB in macrophages [186]. IL-10 also downregulates the production of proinflammatory monokines [187] (see above), inhibiting the nuclear factors NF-κB, AP-1, or nuclear factor–IL-6 [188]. Unlike IL-4, IL-10 can also enhance degradation of the mRNA for IL-1 and TNF-α [189].

Vitamin D$_3$
Vitamin D$_3$ may exert major immunomodulatory effects in RA [190] that are possibly based on the effects on activated synovial macrophages, resulting in positive regulation of the anti-inflammatory cytokines IL-4 and TGF-β [191]. Accordingly, dietary supplementation of vitamin D$_3$ in collagen-induced arthritis suppresses or prevents arthritis [192]. These immunomodulatory effects may directly or indirectly underlie the observation that, in RA, the serum levels of vitamin D$_3$ are inversely correlated with parameters of disease activity [193]. Vitamin D$_3$ supplementation may therefore represent a simple but effective therapy for RA, although it may increase the risk of developing renal concrements [194].

Gene therapy in arthritis
Gene therapy approaches have been applied to counteract IL-1 and TNF-α, cytokines that are predominantly produced by macrophages [182]. Gene therapy with IL-1 receptor antagonist has proven efficacious in a number of experimental arthritis models [182,195,196]. The same applies to therapeutic overexpression of the soluble IL-1 type I receptor–IgG and the type I soluble TNF-α receptor–IgG fusion proteins, although the latter appears less effective [182]. Recently, gene therapy approaches have also been extended to anti-inflammatory cytokines (ie IL-4 [197],

IL-10 [182], IL-13 [104] and TGF-β [198]). An interesting approach of gene therapy is to achieve 'molecular synovectomy', either by expression of herpes simplex virus-thymidine kinase, with subsequent administration of ganciclovir [199,200], or by overexpression of Fas ligand, resulting in synovial cell apoptosis [201,202]. Gene therapy aimed at neutralizing proinflammatory macrophage products, overexpressing macrophage-regulating mediators, or simply eliminating overly activated macrophages therefore carries some promise for the treatment of arthritis [203].

Conclusion
Although it is unlikely that macrophages represent the 'initiators' of the pathogenetic cascade in RA, it is believed that these cells act as amplifiers of local and systemic inflammation, with a direct contribution to matrix degradation. Locally, macrophages are involved in recruitment and activation of inflammatory cells, cell contact, or cytokine-mediated activation/differentiation of neighbouring cells, secretion of matrix-degrading enzymes, and neovascularization. At a systemic level, macrophages amplify disease via the acute phase response network, production of TNF-α, development of bone marrow differentiation anomalies, and chronic activation of circulating monocytes.

Although identifying the diease aetiology remains the only means to fully silence RA, efforts to therapeutically address activated monocytes/macrophages have the advantage of striking the very cell population that mediates/amplifies most of the irreversible cartilage destruction, thus minimizing adverse effects on other cells that may have no (or marginal) effects on joint damage. The development of more sophisticated means to reduce the numbers of activated macrophages (eg by selective apoptosis-inducing agents), to inhibit activation signals and/or their specific macrophage receptors, or to selectively counteract the macrophage products that act as disease amplifiers, raises the hope of optimizing the therapeutic success against joint inflammation and irreversible joint damage.

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Authors' affiliations: Raimund W Kinne and Ernesta Palombo-Kinne (Experimental Rheumatology Unit, Friedrich Schiller University, Jena, Germany), Rolf Bräuer (Institute of Pathology, Friedrich Schiller University, Jena, Germany), and Bruno Stuhlmüller and Gerd-R Burmester (Department of Rheumatology and Clinical Immunology, Charité University Hospital, Humboldt University of Berlin, Berlin, Germany)

Correspondence: Raimund W Kinne, MD, Experimental Rheumatology Unit, Friedrich Schiller University Jena, Bachstr 18, D-07740 Jena, Germany. Tel: +49 3641 65 71 50; fax: +49 3641 65 71 52; e-mail: Raimund.W.Kinne@rz.uni-jena.de