

Review

Targeting monocytes/macrophages in the treatment of rheumatoid arthritis

Jean-Luc Davignon^{1,2,3,4}, Myriam Hayder^{1,2,3}, Michel Baron^{1,2,3},
Jean-Frédéric Boyer^{1,2,3,4}, Arnaud Constantin^{4,5}, Florence Apparailly⁶,
Rémy Poupot^{1,2,3} and Alain Cantagrel^{1,2,3,4}

Abstract

Biotherapies have revolutionized the treatment of RA. However, much work is needed to understand all the mechanisms of these biotherapies, and alternatives are needed to circumvent adverse effects and the high cost of these long-lasting treatments. In this article we outline some of the approaches we have used to target monocytes/macrophages as major components of inflammation and bone homeostasis. We also discuss how anti-TNF- α antibodies target monocytes/macrophages in the complex mechanisms contributing to inhibition of inflammation.

Key words: monocytes, RA, inflammation, novel therapies, biotherapies, targeting, TNF- α , siRNA, cPLA₂ α , dendrimer, animal models.

Introduction

RA presents with multiple manifestations of inflammation that are due to complex causes [1]. Many different cell components are involved in the development of inflammation, including neutrophils, mastocytes, T and B lymphocytes, and monocytes/macrophages. Activation of these cells leads to the production of cytokines and mediators responsible for inflammation. TNF- α has been shown to be the master element of inflammation in RA [2, 3]. Accordingly, therapies aimed at blocking this cytokine have emerged as a major tool in the treatment of RA [1]. Biotherapies have thus revolutionized the treatment of RA [4]. MAbs and soluble receptors are targeting major inflammatory cytokines [5] as well as T- and B-cell populations of the immune system, thus inhibiting inflammation [6].

Monocytes/macrophages are central to the pathophysiology of inflammation [7] as well as atherosclerosis [8]. More specifically, they have been found to be activated in RA [9, 10] and to massively infiltrate inflammatory sites [11], i.e. synovial membranes in RA [12, 13] and produce

TNF- α [13]. This activation has led to the identification of genes activated in monocytes from RA patients [14]. There is an increase of CD14⁺/CD16⁺ monocytes in blood from RA patients [15]. Whether this increase reflects or contributes to the pathogenesis of monocytes/macrophages is unknown. But the increase of soluble CD14 in RA relates to the activation of monocytes/macrophages [16, 17]. The depletion of monocytes using specific antibodies [18] can prevent their presence in the pannus [19] and thus attenuate inflammation.

In addition to their central role in inflammation, monocytes/macrophages are at the origin of pathological bone erosion in RA due to their excessive differentiation into osteoclasts (OCs), which are the only cells specialized in bone resorption [20]. Their differentiation is mediated by two major cytokines, M-CSF and RANKL (receptor activator of nuclear factor κ B ligand). M-CSF binds to its receptor c-FMS, (cellular-feline McDonough strain sarcoma virus oncogene homologue, or CSF-1 receptor, or CD115), which in turn induces the expression of RANK on monocytes. RANKL expression by synovial fibroblasts is induced by pro-inflammatory cytokines such as TNF- α , IL-1, IL-6 and IL-17 [21]. Thus bone homeostasis is modulated by inflammation and immunological events. The concept of osteoimmunology emerged a few years ago to account for the interplay between the bone and immune systems [20, 22]. Monocytes/macrophages are thus ideal targets to influence osteoclastogenesis in inflammation.

Complex cell interactions are involved in all immunological processes. Cytokine stimulation of T lymphocytes

¹INSERM, U1043, ²CNRS, U5282, ³Centre de Physiopathologie de Toulouse Purpan, Université de Toulouse, UPS, ⁴Centre de Rhumatologie, Centre Hospitalier Universitaire de Toulouse, Hôpital Purpan, ⁵INSERM U1027, Toulouse F-31400 and ⁶INSERM U 844, Hôpital Saint Eloi, and Université Montpellier I, Montpellier, France.

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Correspondence to: Jean-Luc Davignon, UMR INSERM 1043, CPTP, CHU Purpan, 1 Place Baylac, 31300 Toulouse, France.
E-mail: jean-luc.davignon@inserm.fr

regulates the production of TNF- α by monocytes/macrophages and modulates their plasticity [23–25]. This may have significance in RA pathogenesis due to the importance of T-cell activation [26]. For example, in RA, monocytes/macrophages from inflamed joints have been shown to induce the development of Th17, thus contributing to the amplification of inflammation [27], and T-monocyte contact is involved in cartilage destruction [28]. These studies exemplify the importance of targeting T-monocyte/macrophage interaction [29]. In this respect, as monocytes/macrophages are considered to be antigen presenting cells, CTLA4-Ig (abatacept) acts by blocking this interaction [26, 30].

Blocking TNF- α was shown to result in the inhibition of IL-1 β , IL-6 and IL-8 production, thus putting TNF- α centre stage of inflammatory cytokine regulation and thus providing a rationale for the use of anti-TNF reagents [3, 31]. TNF- α is a transmembrane protein that needs cleavage by TNF- α converting enzyme (TACE) to be released as a soluble cytokine [32–34]. In monocytes/macrophages, reverse signalling occurs through transmembrane TNF- α (tmTNF- α) and regulates cell-cell interaction [35]. This reverse signalling may also explain some of the effects of anti-TNF drugs [36]. Thus, in addition to blocking soluble TNF- α , anti-TNF reagents may act through the interaction with tmTNF- α [37].

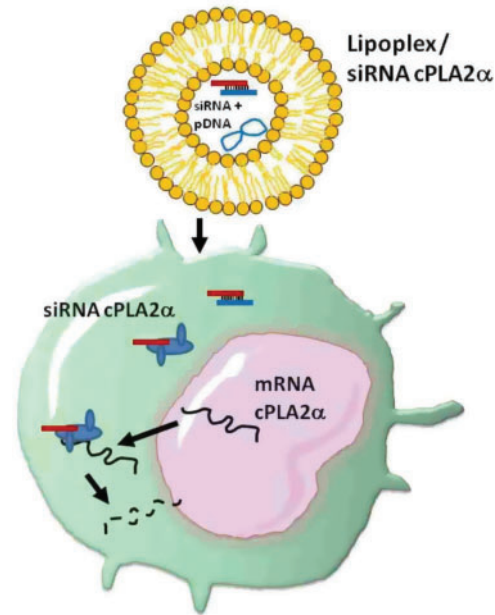
While TNF- α is produced in large quantities by monocytes/macrophages and is a direct effector of inflammation, it can also activate cytosolic phospholipase A₂ α (cPLA₂ α) [38]. This enzyme is strongly expressed in monocytes/macrophages and hydrolyses phospholipids into arachidonic acid with subsequent activation of cyclo-oxygenase and PG synthases [39]. This pathway generates lipids such as PGs and leucotrienes that have been shown to induce and maintain inflammation. Besides inflammation, cPLA₂ α is an important regulator of bone resorption [40].

Targeting monocytes/macrophages should be a powerful way of inhibiting inflammation and bone erosion in arthritis. Their plasticity is a major property that helps the switch from a pro-inflammatory phenotype (M1) to an anti-inflammatory state (M2) [41]. In this review we highlight some of approaches we have used to target monocytes/macrophages and treat experimental arthritis, as well as potential consequences of anti-TNF biotherapy on monocyte/macrophage inflammation and osteoclastogenesis. Identifying molecular targets within this cell population will help in creating new therapeutic solutions.

Inhibition of cPLA₂ α in monocytes/macrophages using lipoplexes as siRNA vectors

Recently a new generation of vectors that target the mononuclear phagocyte system has been developed [42–45]. These vectors, called lipoplexes, allow for the delivery of small interfering RNA (siRNA) specific for various molecular targets. Lipoplexes, as well as other techniques, have been used for the silencing of inflammatory

Fig. 1 Targeting monocyte using lipoplexes.



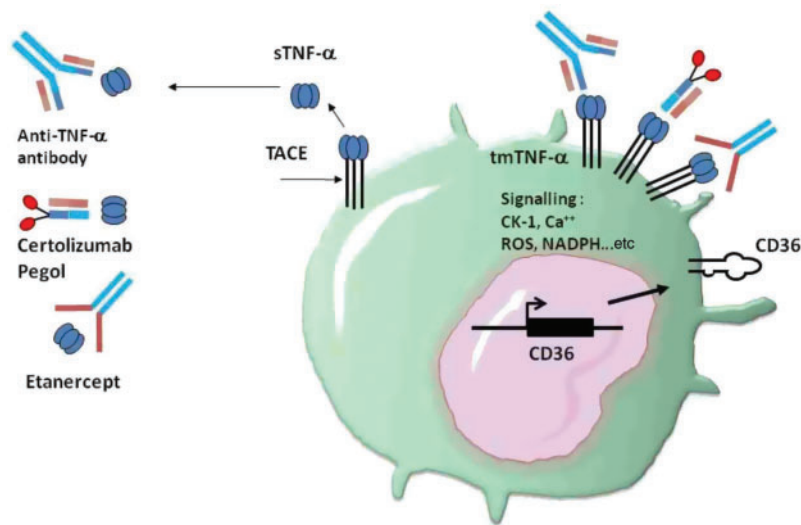
siRNAs are incorporated into lipoplexes combined with plasmid DNA as a carrier. siRNAs are then associated with the Risc complex, leading to degradation of specific mRNA. Specific cPLA₂ α inhibition is depicted.

cytokines [42, 46]. Our approach was to target a specific cytosolic phospholipase A₂, cPLA₂ α , in order to inhibit the cascade involved in the production of PGs in monocytes/macrophages [47]. Among the huge family of phospholipase A₂ enzymes, cPLA₂ α is one of the most expressed and active in monocytes/macrophages [48]. Several papers have pointed out the increase in cPLA₂ α in systemic inflammation and cancer [49, 50]. Inhibiting its activity with small synthetic molecules has helped in finding the importance of cPLA₂ α in arthritis [51] and experimental autoimmune encephalomyelitis [52].

In our published experiments [47], the cPLA₂ α siRNA distribution was associated with a reduced cPLA₂ α expression and activity within spleen monocytes/macrophages and inflamed joints, and pro-inflammatory cytokines such as TNF- α and IFN- γ were also attenuated. Histology showed that there was a near-complete inhibition of cell infiltration in joints from mice treated with cPLA₂ α lipoplexes. This resulted in a significant reduction of arthritis as measured by paw swelling as well as arthritis score. Fig. 1 depicts the lipoplex–siRNA approach.

The gene knock-out approach showed that mice deficient in cPLA₂ α are resistant to CIA [53]. Antisense oligonucleotides have also been used [54]. However, knocking out genes without cell targeting implies blocking specific gene expression in all cells, whatever the cell lineage is [55]. Thus silencing a key producer of pro-inflammatory lipid mediator within a specific cell type such as monocytes/macrophages may help to focus on key effectors

Fig. 2 Illustration of reverse signalling by anti-TNF drugs.



Inhibition of soluble TNF- α as well as of tmTNF- α : induction of CD36 expression as an illustration of reverse signalling through tmTNF- α induced by antibodies (remicade, adalimumab, golimumab) as well as by soluble receptor (etanercept) and pegylated Fab fragment (certolizumab pegol). CK-1: creatine kinase 1; ROS: reactive oxygen species.

and avoid potential side effects by excluding the targeting of other cell types.

Monocyte/macrophage tmTNF- α as a target of biotherapy

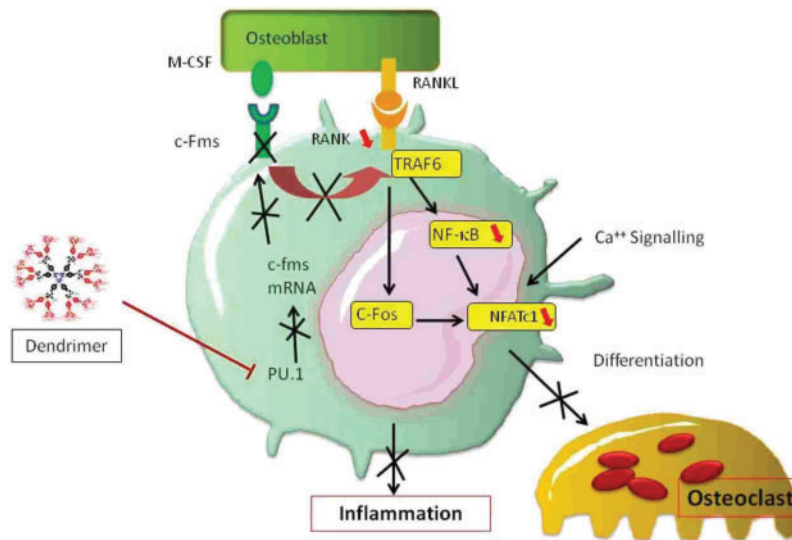
The use of anti-TNF reagents as a biotherapy has massively reduced the burden of inflammation and bone erosion in good responders [56]. However, a proportion of patients remain resistant to anti-TNF treatments [50]. Three major types of anti-TNF drug have been developed: antibody (fully human or humanized), soluble receptor and single immunoglobulin chain [57]. All (infliximab, etanercept, adalimumab, certolizumab pegol, golimumab) bind to soluble TNF. It is generally admitted that blocking soluble TNF- α is the major mechanism for decreasing inflammation. However, since TNF- α exists also as a transmembrane protein, blocking its soluble part may not be the only way to block functional activity of TNF- α with antibodies.

Soluble TNF- α is released from tmTNF- α through cleavage by the enzyme TACE [34]. Processing of the cytoplasmic portion of TNF- α also occurs through protease SPPL2b in dendritic cells [58–60]. The intracellular part of TNF- α migrates to the nucleus, probably due to its putative nuclear localization sequence and signals that involve Ca²⁺ [58]. Again, those signals may be different depending on the anti-TNF. Numerous reports have indicated that tmTNF- α can transmit signals to monocytes/macrophages [37, 58, 59, 61–63]. This is called reverse signalling. Thus therapeutic antibodies can block soluble TNF- α as well as participate in cell signalling through binding to tmTNF- α [64–66].

We have shown that anti-TNF reagents induce an increase of CD36 in monocytes/macrophages. The mechanism involves redox signalling via NADPH oxidase activation [62]. Since CD36 is a scavenger receptor involved in the transport of cholesterol, this increase is relevant to the development of atherosclerosis as a complication of RA [75, 76]. This increase may be related to the lower incidence of atherosclerosis in RA patients treated with anti-TNF [77, 78]. How the increase of CD36 participates in the pathophysiology of atherosclerosis in RA will require clinical studies. Although we have concentrated on CD36, several other markers may be modified in monocytes/macrophages by reverse signalling. This is being investigated in our laboratory. Differences in reverse signalling with anti-TNF drugs may be due to structural differences of various anti-TNFs and may explain disparate adverse effects regarding tuberculosis [79–81] and disparate efficacy in inflammatory bowel disease [36, 63]. These differences could also modulate inhibition of nuclear factor- κ B (NF- κ B) and suppression of IL-1 β responses in monocytes/macrophages [37], as well as induction of Tregs by dendritic cells [82]. A schematic description of various anti-TNF and reverse signalling is depicted in Fig. 2.

Thus targeting monocytes/macrophages with various anti-TNF reagents will result in different outcomes. Predicting these outcomes and understanding how anti-TNF drugs target monocytes/macrophages and orient them towards specific phenotypes may help choose the most appropriate and most beneficial treatment for the patient.

However, cells other than monocytes/macrophages can express tmTNF- α . On T cells, tmTNF- α signalling has been shown to induce E-selectin expression [63, 83, 84].

Fig. 3 Inhibition of OC differentiation and of inflammation by dendrimer ABP.

Dendrimer is internalized by monocyte and inhibits the expression of c-FMS mRNA downstream to PU.1 inhibition. Subsequently RANK and NFATc1 induction is impaired, leading to inhibition of OC differentiation. Production of pro-inflammatory cytokines is inhibited and inflammation is reduced.

On the other hand, blocking tmTNF- α on T cells decreases TNF- α production by monocytes/macrophages [84]. Thus the tmTNF- α pathway is most suitable for the inhibition of monocyte activation.

Inhibition of c-FMS in monocytes/macrophages using phosphorus-based dendrimer

Dendrimers are highly branched tree-like polymers, with precisely defined structure and molecular weight. Their surface multivalency allows for polyvalent interactions with cellular and molecular targets. Phosphorus-based dendrimer aminobisphosphonate (ABP) has been described to possess anti-inflammatory properties [85–87].

The main cellular target of dendrimer ABP has been found to be monocytes/macrophages [88]. Internalization of dendrimer occurs through a rapid process whose molecular mechanism is not yet deciphered. Although the receptors of dendrimer ABP are as yet unknown, some of its molecular targets have been identified [85, 88]. Interaction of dendrimer with monocytes/macrophages induces a decrease of c-FMS cell surface expression as well as mRNA expression [85]. PU.1 expression, which controls c-FMS, is also decreased. Inflammation, arthritis score paw swelling and bone resorption were dramatically reduced in two experimental models, IL-1 ra $^{-/-}$ and K/BxN serum-induced arthritis [85]. More targets are probably to be identified, but the decrease of c-FMS expression can explain at least part of the effects of dendrimer ABP observed on osteoclastogenesis and inflammation.

The interaction of M-CSF with its receptor c-FMS induces a cascade of signals that is indispensable for

osteoclastogenesis [89]. RANK expression is induced by the interaction of M-CSF with its receptor c-FMS, leading to subsequent recruitment of TRAF6 [20]. These signalling events ultimately lead to NF- κ B and nuclear factor of activated T cells (NFATc1) activation mediating inflammation and osteoclastogenesis [20]. Thus targeting c-FMS may be a good way to reduce both inflammation and osteoclastogenesis.

To this end, multiple reagents have been developed: inhibitor of c-FMS kinase imatinib has been shown to promote bone growth in experimental arthritis [90] and bone loss in chronic myeloid leukaemia [91, 92], but it targets platelet-derived growth factor receptor (PDGFR) as well as c-FMS. This may have adverse consequences. Other inhibitors of kinases have been reported [93–95].

An antibody specific for c-FMS has been used in an experimental model of TNF- α -induced bone erosion [96] in lipopolysaccharide-induced osteoclastogenesis [97] and in mouse models of RA [98]. However, other antibodies directed towards c-FMS have been shown to either block inflammation [99] or not [100] in models of peritonitis and lung inflammation. This may depend on the type of mAb. Neutralizing mAb specific for M-CSF reduced the severity of established CIA, and M-CSF-deficient op/op mice were resistant to CIA induction [101]. These reports argue strongly in favour of the importance of the M-CSF pathway in inflammation and osteoclastogenesis. Targeting CSF in inflammation and autoimmunity has been considered [102].

A link will have to be found to connect interaction with a putative receptor for the dendrimer ABP and with its molecular targets. Whether dendrimer ABP modifies the expression of other molecules in monocytes/macrophages needs to be demonstrated. Fig. 3 depicts

the current view of the putative mechanism of action for dendrimer ABP.

Discussion

Monocytes/macrophages link adaptive and innate immunity. Although monocytes/macrophages are central to inflammation, none of the current biotherapies specifically target monocytes/macrophages in RA. Their plasticity [41, 103] makes them an ideal target for the treatment of inflammation, especially arthritis. We have been interested in this cell lineage to search for possibilities for treating arthritis.

Our work has focused on potential experimental therapeutics against arthritis by targeting monocytes/macrophages cPLA₂ α and OC differentiation using, respectively, lipoplexes and dendrimers. Another goal was the understanding of the effect of currently available drugs on reverse signalling in monocytes/macrophages (anti-TNF). The effect of anti-TNF reagents on tmTNF- α will have to be taken into account. Imbalance regarding the production of cytokines by monocytes/macrophages in response to tmTNF- α signalling could account for various phenotypes observed in patients undergoing anti-TNF biotherapy.

Higher expression of tmTNF in monocytes from RA patients compared with healthy donors was reported [37]. A decrease in IL-1 β production by infliximab and an increase in apoptosis by tmTNF were observed in RA compared with healthy donors. Thus reverse signalling is expected to be a potent regulator of signalling in monocytes from RA patients.

Reverse signalling may also explain the absence of granulomatous infections in patients treated with etanercept, whereas this is a complication of treatment with anti-TNF antibodies [36, 58]. Another example of potential reverse signalling is anti-inflammatory response mediated by infliximab but not by etanercept in Jurkat T cells [63].

Gene expression in the blood and synovium from patients treated with infliximab can also be predictive of the response to treatment [67, 71–74]. Again, reverse signalling may account for these modifications. However, the mechanisms responsible for response and non-response to anti-TNF antibody treatment are not fully understood. Anti-infliximab antibodies can be detected in the serum of treated patients. They impair the efficiency of treatment and may be related to the responder/non-responder status of patients [67–70]. However, mechanisms other than neutralization of soluble TNF- α have been suggested to be responsible for the responder/non-responder status [67]. These mechanisms may result from reverse signalling. Experiments are under way to test for the molecular and cellular consequences of various anti-TNF drugs.

When tested in experimental arthritis, siRNA-cPLA₂ α -lipoplexes as well as dendrimers were efficient at reducing inflammation and joint destruction. However, lipoplexes needed to be combined to a specific inhibitory element, siRNA [44, 104]. We used cPLA₂ α siRNA to specifically target the PG pathway in monocytes/macrophages [47]. This resulted in decreased production of TNF- α and IFN- γ ,

but not IL-17, suggesting that the inhibition of PGE2 production was sufficient to resolve arthritis even if IL-17 was not down-regulated. Dendrimer ABP, however, possessed intrinsic activity and was much more effective with regard to the inhibition of inflammatory cytokines such as IL-1, IL-6, IL-17 and TNF- α . Dendrimer ABP was also capable of blocking OC differentiation *in vitro* and *in vivo*, thus indicating that the inflammation/osteoclastogenesis pathways were intertwined and thus dampened together. Other types of dendrimer have also been used as carriers of various compounds [105, 106], but their most remarkable properties rely in directly targeting and modulating the function of cell populations [85, 86] and combating viral infection [107, 108]. Although it is established that the monocyte is the most important target cell population of dendrimer ABP, no data are yet available regarding the cellular receptors. Identifying a receptor may help predict some of the properties of dendrimer ABP on monocytes/macrophages.

Various therapies are targeting T-cell function (CTLA4-Ig, [26]), B cells (anti-CD20, [109]) and cytokines such as TNF- α , IL-6, IL-17 and IL-1. The use of anti-CD20 antibodies has highlighted the role of B cells in the treatment of RA. Its efficacy has been reported to be due at least in part to reduction of the Th17 response [110] and the removal of short-lived autoreactive plasma cells in a mouse model of RA [111]. T and B cells are central to adaptive immunity, but so far, no autoimmune-specific antigen of these cells in RA has been used as a drug target. In particular, although ACPAs are currently used for the diagnosis of RA [112], and are probably involved in the pathogenesis of RA [113], no treatment is yet aimed at these immunological specificities.

In conclusion, although current biotherapies have changed the outcome and complications of RA, alternatives are worth considering. In this respect, monocytes/macrophages are strong candidates as targets in the treatment of RA. Their central role in inflammation and bone homeostasis as well as their plasticity makes them suitable for modulating the cytokine environment in systemic arthritis. Future studies will determine if this approach can be envisaged in other autoimmune diseases as well.

Rheumatology key messages

- Monocytes are strong candidates as targets in the treatment of RA.
- Lipoplexes and phosphorus-based dendrimers target monocytes in arthritis.
- Anti-TNF may have consequences on monocyte biology in RA through reverse signalling.

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