Review

Common inflammatory mediators orchestrate pathophysiological processes in rheumatoid arthritis and atherosclerosis

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RA is characterized by a systemic inflammatory state, in which immune cells and soluble mediators play a crucial role. These inflammatory processes resemble those in other chronic inflammatory diseases, such as atherosclerosis. The chronic systemic inflammation in RA can be considered as an independent risk factor for the development of atherosclerosis, and represents an important field to investigate the reasons of the increase of acute cardiovascular events in RA. In the present review, we focused on several mediators of autoimmunity, inflammation and endothelial dysfunction, which can be considered the most promising targets to prevent atherogenesis in RA. Among several mediators, the pro-inflammatory cytokine TNF-α has been shown as a crucial factor to induce atherosclerosis in RA patients.

KEY WORDS: Rheumatoid arthritis, Cardiovascular, Cytokine and inflammatory mediators, Inflammation, Chemotactic factors.

Introduction

RA is a chronic inflammatory disease, which affects ~1% of the population world wide [1–3]. Its aetiology is still unknown. However, RA is characterized by a systemic inflammatory state, involving several organs, including joints, skin, eyes, lung and blood vessels [4]. Immune cells and soluble inflammatory mediators play a crucial role in RA pathogenesis. Various leucocyte populations, orchestrated by several cytokines, chemokines, growth factors and hormones, infiltrate rheumatoid tissues and increase injury [5]. These inflammatory processes resemble those in other chronic inflammatory diseases, such as atherosclerosis [6]. The activation of monocytes, T and B cells, vascular endothelial cells and the elevation of circulating inflammatory factors and markers characterizing both diseases, suggest that different inflammatory disorders can be induced by common inflammatory processes. In particular, inflammation in RA is now considered as an independent risk factor for the development of atherosclerosis [7–12]. This is suggested by several independent findings, indicating a possible strong association between RA and atherosclerosis. Atherogenesis is accelerated in RA patients [6] and increases the mortality of these patients for acute cardiovascular events [13–16]. The excess of cardiovascular mortality in RA patients could be associated with the long-term corticosteroid treatments against RA [17] or, intriguingly, with the increase of circulating inflammatory cardiovascular factors known to play a crucial role during atherogenesis [18]. Indeed, several soluble mediators of autoimmunity, inflammation and endothelial dysfunction can be considered the most promising targets to prevent atherosclerosis in RA.

Role of dyslipidaemia in the pathogenesis of atherosclerosis in RA

Traditional atherosclerotic risk factors play a crucial role in the development of atherosclerosis in patients with RA. Among Framingham risk factors, an unbalance between levels and activation of lipoproteins contribute to the acceleration of atherosclerosis in RA. In particular, the suggested mechanisms are subsequent to endothelial dysfunction. Increased spaces between altered endothelial cells in RA patients permit the entry of low-density lipoproteins (LDLs) [19]. Once retained in the intima, LDLs are oxidized (OxLDL) and activate endothelial cells to up-regulate adhesion molecules and the chemokine secretion to recruit circulating leucocytes within atherosclerotic plaques [20]. When monocytes/macrophages infiltrate atherosclerotic plaques, they uptake OxLDL and form the 'foam cells' that are considered key players by secreting inflammatory mediators. Subjects suffering from RA have increased levels of native OxLDLs [21]. Furthermore, functional abnormalities of the endothelium have been detected in in various cohorts of RA patients [22, 23]. Given this evidence, OxLDL are pivotal molecules in the development of atherosclerosis in RA. They should be considered a crucial pro-inflammatory stimulus in the vicious circle, which sustains chronic inflammation in RA. New therapies targeting the modulation of lipid profile in RA have been investigated with controversial results [24, 25]. On the other hand, high-density lipoproteins (HDLs) have been shown to exert anti-inflammatory activities in both acute and chronic diseases [26]. Dimished levels of HDL have been detected in RA patients [27]. Therefore, the increase of HDL concentrations in RA could ameliorate both disease activity and the associated atherosclerosis. A treatment with an apolipoprotein A-1 mimetic peptide in combination with pravastatin has inhibited CIA [28]. Lipid levels should be monitored in patients with RA to minimize the cardiovascular disease. Further studies are needed to determine the impact of specific lipoprotein particles, small dense LDL and subfractions of HDL on long-term risk of atherosclerosis in RA [29].

Rheumatoid autoimmunity and atherosclerosis: can autoantibodies induce atherosclerosis?

Autoantibody production is a condition strongly associated with RA. Little is known about autoantibodies and atherosclerosis in both humans and animal models [30]. Although not only specific for RA [31], RF increases the risk of developing both RA and atherosclerosis [32, 33]. A recent study also showed an association between autoantibodies against OxLDL and cardiovascular disease in RA [34]. Unfortunately, in these studies the authors did not investigate the autoimmune molecular mechanisms. However, these studies represent a good starting point for future investigations targeting autoantibodies (Fig. 1). The association between aCLs and atherosclerosis have also been investigated and

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probably will be a very promising field of research in the future [35, 36]. Endothelial cells could be the main target for autoantibodies [37–42]. No data are available for antibodies against citrullinated proteins, which are specific and predictive for RA [43].

Rheumatoid inflammatory mediators and atherosclerosis

CRP

CRP is a member of the pentraxin family, first described in 1930 by Tillet and Francis [44] in the sera of patients suffering from pneumonia. Mainly produced by the liver, CRP was considered for many decades as a low, specific systemic marker of inflammation. Recently, it has been shown that several cell types are capable of secreting CRP in inflammatory microenvironments, such as rheumatoid synovium and atherosclerotic lesions (Fig. 2) [45–47]. In these inflamed tissues, CRP directly activates immune cells with the secretion of other inflammatory molecules, by initiating a vicious circle that maintains and increases the inflammatory state [48]. This experimental evidence strongly supports CRP as an active inflammatory mediator with both systemic and local effects. In addition, this may suggest that inflammatory disorders, characterized by high levels of CRP, can develop a secondary immune cell activation, which may result in the increase of atherogenesis. Therefore, the chronic increased CRP serum levels in RA patients [49] can directly induce an acceleration of atherosclerosis and its complications [50, 51]. Numerous prospective epidemiological studies showed that in healthy subjects, serum CRP predicts myocardial infarction mortality [51–53], stroke [54–56] and arrhythmias, including sudden cardiac death [57]. A meta-analysis of 14 prospective long-term studies showed that after correction for age, smoking and other cardiovascular risk factors, CRP was strongly related to coronary heart disease [58]. These studies show that CRP should be considered a direct pro-inflammatory factor in the pathogenesis of inflammatory diseases such as RA and atherosclerosis.

TNF-α

TNF-α is a classical pro-inflammatory mediator and a member of a cytokine family including Fas ligand and CD40 ligand. TNF-α induces deleterious effects in several inflammatory diseases through the binding with two different receptors (called types I and II), which are expressed in all cell types except erythrocytes [59]. This suggests that TNF-α, as CRP, can mediate both local and systemic responses during inflammatory diseases (Figs 3 and 4). RA as well as atherosclerosis represents an inflammatory disorder in which TNF-α play a crucial role. This is strongly supported by studies in both humans and animal models.
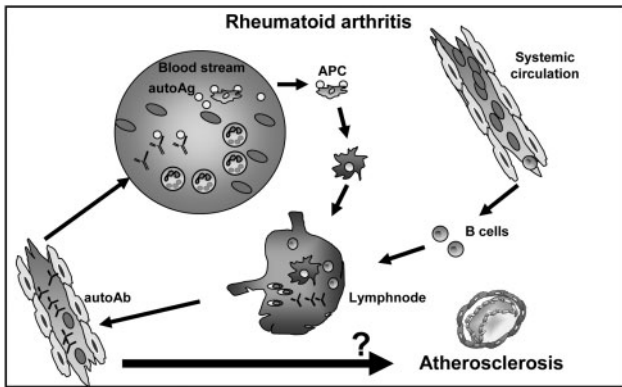

FIG. 1. Autoantibody production in RA subjects could increase atherosclerosis. Auto-antigens (autoAg) are captured in the blood stream, and then presented to B lymphocytes by antigen-presenting cells in the lymphnode. Here, B lymphocytes differentiate to plasma cells and produce autoantibodies (autoAb), which might be involved in increasing atherosclerosis in RA.
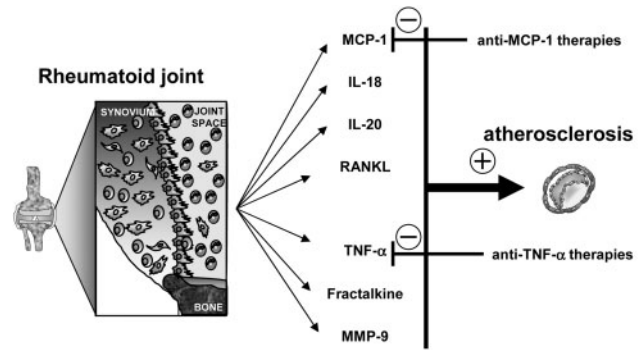

FIG. 3. Rheumatoid joints secrete pro-inflammatory soluble factors, which could accelerate atherosclerosis. Several mediators, released in the blood stream by the inflamed joints, accelerate atherosclerosis in RA patients. Among these, TNF-α is the most promising target to reduce athersoclerosis associated with RA.
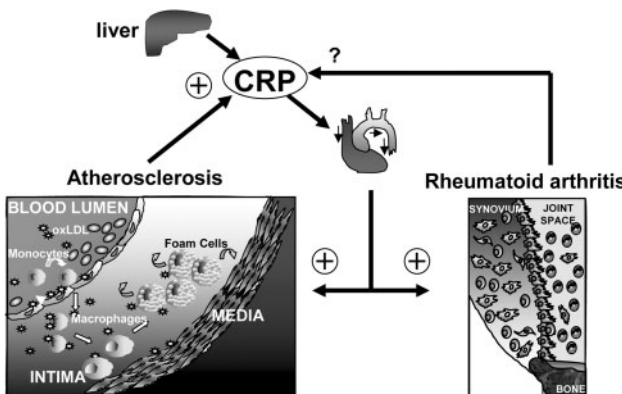

FIG. 2. CRP increases both atherosclerosis and RA. CRP is mainly secreted by hepatocytes in the blood stream. CRP increases immune and vascular cell functions and tissue inflammation. Inflammatory cells in synovium and atherosclerotic plaque further produce CRP by increasing CRP-mediated local inflammation.
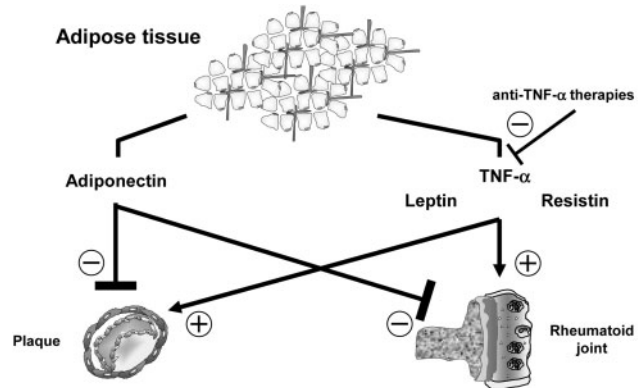
FIG. 4. Adipose tissue produces pro- and anti-inflammatory adipocytokines involved in both RA and atherosclerosis pathophysiology. Adiponectin is considered one of the most promising natural anti-inflammatory mediators against RA and atherosclerosis. The other adipocytokines are currently under investigation, with still controversial results in the regulation of inflammatory processes. The majority of publications consider these adipocytokines as 'pro-inflammatory', rather than 'anti-inflammatory'.

Mouse models of arthritis have also been developed independent of TNF-α [60–62]. However, blockade of TNF-α activity has been shown to influence both disease and inflammatory cells in mice [63, 64]. In humans, the direct positive association between serum levels of TNF-α with the activity of RA [65]. In addition, clinical improvements obtained in several clinical trials targeting TNF-α indicate a promising therapeutic strategy [66]. During athero-sclerotic complications, such as myocardial ischaemia, TNF-α plasma levels have been shown to be very high [67–70]. The inflammatory cascade mediated by TNF-α is quite similar in RA and atherosclerosis, suggesting a deleterious role of this cytokine in both diseases. TNF-α may induce atherosclerosis in RA patients by interfering with various processes. It not only activates inflammatory and endothelial cells [71, 72], but also induces pro-thrombotic states, insulin resistance and dyslipidaemia [72]. Accordingly, anti-TNF-α treatment has been shown to increase HDL cholesterol [73–75] and improve insulin resistance [76, 77] and, transiently also endothelial dysfunction [78, 79]. Although the benefits in endothelial function induced by anti-TNF-α treatments are still controversial [80, 81], improvement of the other aforementioned conditions have to be considered a crucial contribution in the development of secondary atherosclerosis in RA patients [82]. However, the absence of analysis of acute cardiovascular events as clinical end-points in clinical trials with anti-TNF-α treatments is still the strong limitation of the benefits of these therapies in rheumatoid-associated atherosclerosis (Fig. 3) [83–85]. The assessment of cardiovascular global risk, by using serum markers or other indicators, is clearly not sufficient to propose new treatment indications in order to to improve the atherosclerotic burden in RA patients. Therefore, in the future the most important field of investigation for anti-TNF-α treatments for rheumatoid patients should be focused not only on improving joint symptoms, but also on reducing cardiovascular disease burden for these patients.

The RANK ligand (RANKL)/RANK/osteoprotegerin (OPG) axis

The RANKL/RANK/osteoprotegerin (OPG) system is a crucial molecular mechanism in the bone resorption and joint destruc-tion in RA [86]. OPG is a natural decoy for RANKL, which inhibits RANKL binding with its cognate receptor RANK on the cell surface by preventing osteoclast differentiation and, thus, reducing bone resorption. Several cytokines, including IL-1 and TNF-α, have been shown to regulate this system [87–89]. Recent evidence suggests that RANKL and OPG balance is also crucial in atherosclerotic plaque calcification, a condition which is diffused in long-standing RA patients [90] and favours plaque rupture [91, 92]. The role of RANKL and OPG in plaque calcification has also been shown in knockout mouse models [93–95]. Circulating RANKL induces plaque instability in humans by inducing monocyte chemoattractant protein-1 (MCP-1) and MMP produc-tion [96]. On the other hand, serum levels of OPG are increased in RA patients and independently associated with coronary artery atherosclerosis [97]. These studies indicate that RANKL/OPG could represent a very important molecular field of investigation to better understand the increase of cardiovascular risk in RA. The strongest limitation for the clinical use of these markers is represented by their poor specificity. However, the RANKL/RANK/OPG axis could be a promising target for future therapies. In this context, experimental data in animal models have provided the first evidence for the therapeutic use of OPG as a possible pharmacological agent to reduce arterial calcification [98]. On the contrary, human data have suggested a direct relationship between increased OPG serum levels and plaque destabilization. This may imply that elevated OPG levels could be compensatory rather than causational in atherosclerotic calcification. Further clinical investigations with large numbers of patients are required

to better clarify the role of serum sRANKL and OPG in RA-induced atherosclerotic plaque calcification.

Adipocytokines

Since the discovery of leptin in 1994 [99], white adipose tissue (WAT) has been found to secrete several inflammatory mediators, which have been called 'adipokines' or 'adipocytokines' (Fig. 4). These molecules orchestrate via endocrine, paracrine, autocrine and juxtacrine mechanisms, and both physiological and physio-pathological processes, including food intake, insulin sensitivity, immunity and inflammation [100, 101]. Adipocytokines induce their activities through the binding to selective transmembrane receptors on different cell types. At present, the most studied adipocytokines are adiponectin, leptin, resistin, visfatin and also TNF-α. Leptin is a non-glycosylated peptide hormone, encoded by the gene obese (*ob*) in mice and by the gene *LEP* in humans [99]. In animal models, its synthesis is regulated by food intake, sex hormones and inflammatory mediators, and its levels are negatively correlated with glucocorticoids and positively with insulin [102–105]. The role of sex hormones is also confirmed by studies performed in humans, showing that leptin levels are higher in women than in men [106]. Leptin levels have also been found increased in humans in several inflammatory diseases, including obesity, metabolic syndrome, RA and atherosclerosis [107–109]. Direct pro-inflammatory activities of leptin on immune response have been shown in human and murine macrophages [110, 111], human neutrophils [112, 113], NK cells [114], dendritic cells [115], T lymphocytes [116, 117] and synovial fibroblasts [118]. Accordingly, leptin-deficient mice, which suffer from thymus atrophy, are immunodeficient animals [119] and are less prone than non-leptin-deficient mouse to develop inflammatory disease [120]. On the basis of these studies, leptin has been investigated as a marker of disease activity in RA patients. On this regard, controversial results have been published [121–124]. Furthermore, anti-TNF-α antibody treatment with adalimumab did not have any effect on serum levels of leptin in RA patients [125]. Therefore, although a crucial role of leptin in inflammatory processes has been shown in humans and animal models [126], further investigations are needed to better understand its active role in RA and associated atherosclerosis. Probably, leptin half-life and consumption in rheumatoid joints could be the most promising field of investigation [127]. Recently, other pro-inflammatory adipocytokines have also also discovered. In humans, resistin is secreted by adipocytes and macrophages, while in rodents it has been identified in WAT and haematopoietic tissues [128]. However, resistin seems to play different roles in humans and rodents. In humans, resistin has been shown to induce pro-inflammatory activities on immune cells in chro-nic inflammatory diseases, including RA and atherosclerosis [129–133]. In RA patients, resistin serum levels have been found increased and associated with higher levels of IL-1Ra [134, 135]. Accordingly, anti-TNF-α therapy rapidly reduces resistin serum levels, indicating that this cytokine is involved in the regulation of resistin secretion [136]. On the other hand, although the injection of resistin into mice joints induces an arthritis-like condition [130], other studies indicate that the initial enthusiasm for animal disease model should be limited [137]. The main reason is that resistin levels depend on both nutritional state and hormonal environ-ment. On the contrary, in murine models of atherosclerosclerosis, resistin has been detected in sclerotic lesions and its level has been found correlated with the severity of the lesion [133]. Therefore, further studies are needed to investigate the role of restitin in atherosclerosis acceleration in RA. Visfatin, apelin, vaspin and hepcidin are the most recently discovered adipocytokines [137]. Their physiological and pathophysiological roles in chronic inflammatory diseases are currently unclear and further investiga-tions are needed. On the contrary, the adipocytokine adiponectin is considered one of the most promising targets against chronic

inflammatory diseases, including atherosclerosis and RA. Adiponectin is prevalently produced in WAT and has been shown to induce anti-inflammatory activities in both humans and animal models. The ablation of the adiponectin gene induces a dramatic insulin resistance in mice under high-fat or high-sucrose diet [138]. This pro-diabetic condition in combination with the increased fatty acid levels and increased proliferation of vascular cells strongly suggests that hypoadiponectinaemia induces a pro-atherogenic state in mice [139]. A direct anti-inflammatory activity of adiponectin has also been shown in humans [140, 141]. Basic research and clinical studies suggest that adiponectin could reduce atherosclerosis in both humans and animal models and should be considered a promising target for anti-atherosclerotic therapies [142–144]. The crucial role of adiponectin in RA also suggests a possible pathophysiological trigger of atherosclerosis in arthritic patients and animal models [145, 146]. Anti-TNF-α therapies have already shown to increase adiponectin levels in RA patients [147–150]. Further studies in the future will probably clarify whether therapies increasing adiponectin levels will be able to reduce the acceleration of atherosclerosis in RA.

CD40 ligand

CD40–CD40 ligand (CD40L) interactions are crucial in both RA and atherosclerosis pathophysiology [151, 152]. Therefore, CD40 could represent another common pro-inflammatory trigger by which RA accelerates atherosclerosis. CD40 has been shown on B cell, dendritic cell, monocyte, macrophage, mast cell, fibroblast and endothelial cell membranes. It regulates several immune functions, such as the B-cell response, antigen-presenting cell activity, monocyte migration and survival [153–155]. Also, platelet activation is induced by CD40–CD40 ligand interactions [156]. Although CD40L can also mediate inflammation independently of its cognate receptor CD40 [157], their binding remains a crucial event in triggering immune cell functions in both humans and animal models [158, 159]. CD40 binds with two forms of ligand. The first form (CD154) is expressed on activated T- and other immune cell membrane, while the second one is a soluble form, called soluble CD40 ligand (sCD40L) [155]. The soluble form is of particular interest because it has been shown as a serological prognostic factor in coronary and cerebral vascular diseases [160]. Furthermore, elevated levels of sCD40L in serum of patients with systemic autoimmune diseases have been shown [161]. After the binding with CD40 ligands, CD40 can be internalized. Depending on the cell type, the intracellular signal is transduced through different pathways, involving TNF receptor-associated factors (TRAFs) [162] and several kinases [163, 164]. The activity of CD40 ligands is considered pro-inflammatory in the majority of cell types expressing CD40. Therefore, blocking CD40–CD40L interactions and the modulation of the downstream intracellular signal transduction represent a promising target against inflammatory disorders [165, 166]. Several pharmacological agents have been shown to reduce CD40L levels both *in vivo* and *in vitro* [167]. Furthermore, anti-CD40L antibody treatment has been shown to increase atherosclerotic plaque stability [168] and limit both atherogenesis [158] and the evolution of established atherosclerosis in mice [159]. The use of mAbs anti-CD154 (the form of CD40L expressed on cell membranes) could represent a powerful tool in the treatment of both RA and atherosclerosis [169, 170]. Phase I/II trials of anti-CD40L antibody treatments in humans with lupus nephritis have shown some positive results [171]. However, the increase of thrombotic events has temporarily stopped these studies in humans. Other clinical studies with the administration of antibodies better tolerated are needed to evaluate a possible modulation in RA-induced atherosclerosis.

IL-18

IL-18 has been originally identified as an IFN-γ-inducing factor in Kupffer cells and macrophages [172]. More recently, IL-18 has been shown as a crucial inducer of IFN-γ secretion in T lymphocytes, NK cells [173, 174] and Th1 [175–177]. Several immune diseases, such as juvenile idiopathic arthritis and RA, have been found associated with high levels of IL-18 (Fig. 3) [178–181]. At present, the molecular mechanisms by which IL-18 induces pro-inflammatory activities are under investigation. A recent paper demonstrated that IL-18 induces not only IFN-γ, but also serum amyloid A (SAA) protein production from rheumatoid synovial fibroblasts [182]. Although the molecular pathways remain unknown, other works showed a clear association between IL-18 levels and atherosclerosis. IL-18 is highly expressed in mouse atherosclerotic lesions [183]. The progression of atherosclerosis is reduced in IL-18-deficient ApoE knockout mice [184]. In addition, serum levels of IL-18 are strong predictors of cardiovascular death in stable and unstable angina patients and are positively associated with carotid intima-media thickness [185–187]. For these reasons, the increase of IL-18 in RA patients could contribute to the acceleration of atherosclerosis.

IL-20

IL-20 is a cytokine discovered in 2001 [188] and belonging to the IL-10 family [189]. Although 28% of amino acid sequences of IL-20 are identical to IL-10, crystallographic analysis shows that IL-20 and IL-10 form different structures (IL-20 is a monomer, while IL-10 is an intercalating dimer) [190]. These structural characteristics could partially explain the different functions of these two cytokines. Cytokines belonging to the IL-10 family exhibit substantial sharing of IL-20 receptor complexes (IL-20R1 and IL-20R2), by increasing the well-known cytokine redundancy [191]. Despite this reduced selectivity for its two receptors, several pro-inflammatory activities and clinical implications of IL-20 have been shown in inflammatory disorders. In a recent study, detection of IL-20 was increased in both inflamed synovium and plasma of patients with RA (Fig. 3) [192, 193]. Also in SFs, IL-20 levels were higher than in controls [192]. This suggests that IL-20 is secreted by macrophages and synovial fibroblasts within rheumatoid tissue and also released in the circulation as a systemic factor. IL-20 also induces local pro-inflammatory activities in inflamed synovium. In fact, in an autocrine manner IL-20 promotes the secretion of other inflammatory mediators by fibroblasts [192]. The role of IL-20 in atherosclerosis is still unclear [194]. Increasing evidence suggests that IL-20 induces atherosclerosis through two different mechanisms shown in mice and humans. First, as a direct autocrine mechanism, IL-20, secreted by macrophages localized in atherosclerotic plaques, induces a local promotion inflammation in mice [195]. This was observed in Apolipoprotein E-deficient mice. On the other hand, IL-20 promotes atherosclerosis through an endocrine systemic pathway. This is an indirect mechanism, secondary to IL-20 release from local inflammatory sites, such as rheumatoid synovium or already advanced atherosclerotic plaques. IL-20 in the circulation induces endothelial cell proliferation, with an increase of neovascolarization in human unstable plaques [196–198]. Therefore, IL-20 secreted within atherosclerotic plaques or released in the circulation, contributes to the development of atherosclerosis and could be a very promising target for modulating both RA and atherosclerosis.

MCP-1

MCP-1, also called CCL2, is a well-known CC chemokine and a classical chemoattractant for monocytes [199]. Recent studies showed that MCP-1 is also capable of attracting CD45RO+ T lymphocytes [200] and NK cells [201]. Furthermore, MCP-1 is

also a potent histamine-releasing factor [202], while its activity on dendritic cells remains controversial [203, 204]. This evidence support the relevant role of MCP-1 during inflammatory processes. Both RA and atherosclerosis, which are characterized by mononuclear cell infiltrates, are pathological disease models to evaluate pro-inflammatory activities of MCP-1 [205–207]. Mice deficient for either MCP-1 or its cognate receptor (CCR2) develop less atherosclerosis [208, 209]. In rats, treatment with blindarit (an inhibitor of MCP-1) improved the course of adjuvant arthritis [210]. In additon, MCP-1 serum levels in humans have been associated with the incidence of coronary artery disease in the general population, and with the clinical symptoms of JRA [211–213]. On the basis of this evidence, MCP-1 should be considered a potent RA and atherosclerotic factor and a target for selective therapies (Fig. 3). Few clinical studies have already been performed. For instance, pioglytazone has been shown to inhibit stent restenosis in atherosclerotic rabbits through the reduction of MCP-1 [214]. A direct demonstration of the benefits of MCP-1 inhibition in atherosclerosis has been performed by using antibodies anti-MCP1 or anti-MCP-1 gene therapies (Fig. 3) [215, 216]. However, much remains to be studied in RA, since the first clinical trial using an anti-MCP-1 monoclonal antibody in humans did not result in clinical or immunohistological improvements [217].

Fractalkine

Among the four chemokine families, CXC3C-chemokine family contains only one member that is called fractalkine or alternatively CX3CL1 [218]. Fractalkine has been shown to play a pro-inflammatory role in the pathogenesis of RA [219]. This is supported by both *in vitro* and *in vivo* evidence. Fractalkine and its cognate receptor CX3CR1 are up-regulated in several inflammatory cell populations in RA patients (Fig. 3) [220–223]. Furthermore, in adjuvant-induced arthritis rats fractalkine has been found crucial in monocyte chemotaxis within inflamed joints [224]. This study was also confirmed by a more recent work, which showed a significant improvement in murine CIA when fractalkine was inhibited [225]. In addition, two clinical studies showed that serum levels of soluble fractalkine correlate with disease activity of RA and are not influenced by anti-TNF-α antibody treatment in humans [226, 227]. Therefore, strong evidence supports fractalkine as a pivotal agent in the pathogenesis of RA pathogenesis, independently on TNF-α. On the other hand, growing evidence also suggests that fractalkine may also be involved in atherosclerosis. In fact, high levels of fractalkine mRNA has been detected in atherosclerotic lesions [228]. Furthermore, fractalkine increases CD8$^+$ T lymphocyte and monocyte recruitment within the plaque [229, 230]. In addition, gene polymorphisms of CX3CR1 have been associated with the increase of coronary artery disease [231]. In contrast, polymorphisms of CX3CR1 do not influence peripheral artery disease [232]. These findings suggest that fractalkine/CX3CR1 interactions may increase both coronary artery disease and RA. Further studies are needed to evaluate the role of fractalkine in RA-induced acceleration of atherosclerosis.

MMP-9

MMPs are proteolytic enzymes, which regulate the cell–matrix composition [233, 234]. The main substrates of MMP-9 are denatured collagen (gelatins) and type 4 collagen, which are the pivotal components of the basement membranes. Monocytes and lymphocytes, activated by cytokines, chemokines, eicosanoids and peptidoglycans [235], secrete MMP-9 to cleave basement membranes and enter into the inflamed tissues. MMP-9 is secreted as an inactive pro-enzyme (called zymogen), which is activated by the removal of a domain, which renders the Zn site able for hydrolysis. MMP-9 activation is a crucial mechanism of tissue injury in several inflammatory diseases, including RA (Fig. 3) [236]. Inhibitors and activators regulate MMP-9 activation [237]. An imbalance between MMP and its tissue inhibitors of metalloproteinases (TIMPs) leads to excess of activated MMP, which results in an increased cartilage degradation. This local activity is also supported by the systemic effects of MMPs. In fact, serum levels of MMP have also been related with the severity of progression of RA [237]. Rheumatoid synovium has been proposed as the main source of MMP-9, which is released in SF and blood circulation [238]. Further investigations are needed to evaluate the complex MMP activity systems, with respectively, inhibitors and activators. An imbalance between these factors is thought as a crucial step during atherosclerotic plaque formation and plaque stability. Expression of MMP-9 mRNA and protein in unstable plaques has been found much higher than in stable plaques in both humans and mice [239–241]. This increase of MMP-9 in unstable plaques is in accordance with the increased infiltration of cells responsible for its secretion, such as macrophages and T lymphocytes [240]. MMP-9 reduces plaque stability by the degradation and digestion of the matrix components of the fibrous cap and by increasing neovascularization [242, 243]. These studies clearly indicate that MMP-9 should be considered as an important factor in atherosclerotic plaque formation in RA patients. Therapies aimed at reducing or increasing the expression of MMP-9 inhibitors may serve as promising options in these patients. In this case, corticosteroids, statins and the intravenous infusion of gamma globulins have been already shown to decrease the amounts of MMP-9 [244–247]. Clinical trials are needed to validate these therapies.

Sex hormones

RA and atherosclerosis are inflammatory diseases influenced by hormonal profile [248, 249]. Oestrogens are considered crucial players in both diseases, by regulating both immune system and lipid profile [250, 251]. Oestrogens bind two receptors, called oestrogen receptor (ER)α or ERβ, and, as a dimer, enhance gene promoters in several cell types [252]. Oestrogens modulate several functions in immune cell, including white blood cell recruitment at inflammatory sites, endothelial nitric oxide (NO) production, MMPs and acute-phase protein production [253]. However, these inflammatory processes regulated by oestrogens do not give an explanation for the clinical association between RA and atherosclerosis. In fact, the female hormone profile should prevent atherosclerosis and increase the risk of RA [254]. However, atherosclerosis has been found accelerated in pre-menopausal female patients with RA [255]. This condition clearly suggests that accelerated atherosclerosis in RA is a multifactorial process. Animal models are needed to better clarify the role of oestrogens in accelerated atherosclerotic processes characterizing RA [256, 257].

Insulin

Other hormones with a possible role during atherogenesis have been found increased in RA patients [258–260]. Indeed, insulin could be considered as a crucial factor in RA-induced atherosclerosis acceleration. Insulin is an anabolic essential hormone for the maintenance of glucose homeostasis, tissue growth and development [261]. It is secreted by the pancreatic β cells, mainly through two distinct rhythms, called 'extrinsic' (in response to meals) or 'intrinsic' (with periods of 5–10 min and 60–120 min, in the absence of food intake) [262]. Rhythm alterations, mainly due to defects on insulin secretion or insulin properties, characterize the development of glucose intolerance and the different types of diabetes mellitus [262]. Glucose intolerance has been found associated with the levels of acute-phase reactants in RA [263]. In these patients, glucose intolerance is mainly due to the unbalance of two different mechanisms: (i) the increase of peripheral insulin resistance, a pro-atherosclerotic

condition, which is mediated by pro-inflammatory cytokines (mainly TNF-α and the other adipocytokines) and free fatty acids; and (ii) the use of corticosteroid therapy, which induces iatrogen diabetogenic effects [264–267]. Surprisingly, immunosuppressive therapy with corticosteroids has been also shown to reduce insulin resistance [263]. This suggests that insulin resistance in RA is mainly caused by the inflammatory mediators. Insulin resistance has also been associated with the increase of cardiovascular disease [268, 269]. Insulin or insulin-like growth factor (IGF)-1 increase atherosclerosis in humans by the direct induction of pro-inflammatory activities on leucocytes, endothelial cells and vascular smooth muscle cells [270–273]. These studies clearly
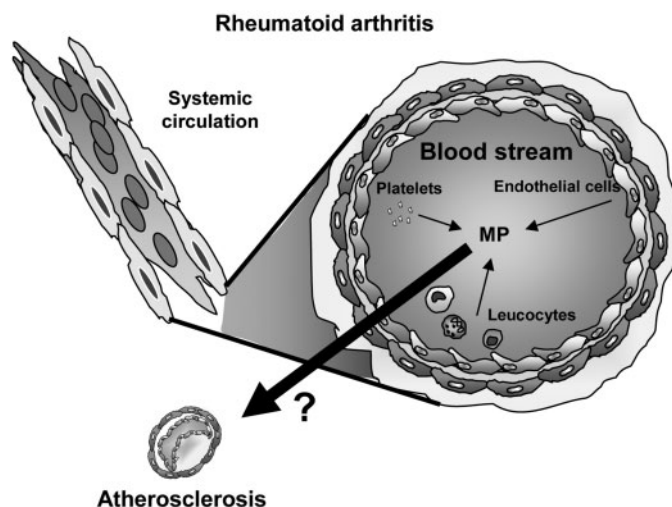

FIG. 5. MP involvement in RA and atherosclerosis. MPs from platelets, endothelial cells and leucocytes are currently under investigation for a possible role in atherosclerosis and RA. The role of platelet MP in atherosclerosis acceleration in RA is the most promising field for researches. MP: microparticles.

indicate that insulin could be a promising prognostic marker for therapies targeting soluble inflammatory mediators in RA. At present, anti-TNF-α therapies have been shown to reduce insulin resistance in RA patients [274–277]. Further experimental evidence is needed to show if the increase of insulin sensitivity could reduce atherosclerotic processes in RA patients.

Rheumatoid-induced endothelial dysfunction and atherosclerosis: adhesion molecules

Endothelial dysfunction is considered as an early step in the initial phases of the atherosclerotic process [278]. The endothelium is a physical barrier between the blood and the intima of vascular wall, essential for the maintenance of vascular homeostasis. Endothelial cell activation and dysfunction are the results of systemic autoimmune processes, in which autoantibodies could play a crucial role. In RA patients, a marked decrease in arterial compliance (measured as pulse-wave analysis) has been showed in the absence of traditional cardiovascular risk factors [279, 280]. In addition, soluble biomarkers of endothelial dysfunction, such as vascular cell adhesion molecules (VCAM)-1, intercellular adhesion molecule (ICAM)-1 and endothelial leucocyte adhesion molecule (ELAM)-1, are increased in RA patients in comparison with healthy controls [281]. The molecular mechanisms, that generate endothelial dysfunction in RA patients, are still unclear. Innate immune system and circulating endothelial progenitor cells have also been investigated, respectively, in mice and humans, but at present, more evidence is needed to support their implications in atherosclerotic processes [282, 283]. The main contribution appears to involve autoantibody activities, but much remains to be clarified.

Other mediators: microparticles

MPs are small (0.1–1 μm) membrane-bound vesicles circulating within peripheral blood, which recently have been shown to be

TABLE 1. Role of inflammatory mediators in RA and atherosclerosis

Factor	Functions	RA	Atherosclerosis
Lipoproteins	Humans: Lipid profile regulates inflammation Animal models: Lipids increase mainly atherosclerosis	LDL: increase HDL: decrease	LDL: increase HDL: decrease
Autoantibodies	Humans: possible activity against endothelial cells. Animal models: no direct evidence in pathophysiology	Probably increase	Probably increase
CRP	Humans: pro-inflammatory activity in immune cells. Animal models: pro-inflammatory in rabbits	Increase	Increase
TNF-α	Humans: pleiotropic inflammatory activity (except red cells) Animal models: pro-inflammatory.	Increase	Increase
RANKL	Humans: increase of plaque instability Animal models: increase of vascular calcification	Increase	Increase
Adiponectin	Humans: anti-inflammatory in vascular and immune cells Animal models: anti-inflammatory in immune cells	Decrease	Decrease
Other adipocytokines	Humans: up regulation of leucocyte functions Animal models: increases inflammation (except resistin)	Increase	Increase
CD40L	Humans: regulation of immune cell functions and survival Animal models: increase of leucocyte recruitment	Increase	Increase
IL-18	Humans: induction of cytokine secretion in T cells Animal models: it is expressed in inflamed tissues	Increase	Increase
IL-20	Humans: neovascularization in inflamatory tissues Animal models: promotion of inflammation in tissues	Increase	Increase
MCP-1	Humans: chemoattractant for monocytes, T cells Animal models: activation of immune cells	Increase	Increase
Fractalkine	Humans: chemoattractant for lymphocytes Animal models: chemoattractant for monocytes	Increase	Increase
MMP-9	Humans: plaque instability and tissue damage Animal models: plaque instability and tissue damage	Increase	Increase
Sex hormones	Humans: oestrogens modulate immune cell functions Animal models: anti-inflammatory effects *in vitro*	Increase	Decrease
Insulin	Humans: increase of leucocyte functions Animal models: increase of vascular inflammation	Increase	Increase
Adhesion molecules	Humans: increase of leucocyte recruitment in tissues Animal models: increase of leucocyte rolling and migration	Increase	Increase
MP	Humans: platelet MP increase leucocyte aggregation Animal models: platelet MP increase leucocyte arrest to inflamed endothelium	Not known	Probably increase

associated with thrombotic and inflammatory diseases in humans and mice [284–286]. Because of their small size, MPs quickly circulate in the blood stream and induce potent pro-inflammatory activities, through the binding to residual receptors and ligands expressed on their membrane surface (Fig. 5). Platelet MPs are the most dangerous, because they favour monocyte survival and adhesion to endothelial cells [287]. Platelet MPs also induce leucocyte aggregation to other leucocytes [288] and secretion of IL-1β [289]. On the other hand, T-cell-derived MPs may induce macrophage apoptosis [290]. On the basis of these premises, RA and associated atherosclerosis represent an important disease model, in which mainly platelet MP can induce injury. Platelet MPs have been found to be elevated in plasma and correlated with disease activity in RA patients [291]. Platelet MPs are also detected in SFs of RA patients, although granulocyte and monocyte MPs are predominant here [292]. Within inflamed joints, MPs promote hypercoaguability and synovial activation, and thus favour articular destruction [293]. In the blood stream, increased levels of MPs have been associated with atherosclerosis. In this case, mainly endothelial MPs have been found elevated in acute complications of atherosclerosis, such as acute coronary syndromes [294, 295]. Therefore, RA and atherosclerosis appear to be associated with the increase of different MPs, derived from different cell types. Further studies are needed to investigate in more detail a possible clinical role of MPs in these associated diseases.

Conclusions

Clinical studies showed that RA is a condition that accelerates atherosclerosis. The strong association between these chronic inflammatory diseases is probably linked to common inflammatory processes and hormonal profile (Figs 2–5). Emerging therapeutic strategies for reducing the cardiovascular risk in RA are under investigation [296–300]. Among several mediators (Table 1), cytokines (mainly TNF-α) and chemokines represent the most promising therapeutic targets to reduce atherosclerosis and its complications in RA patients [301]. Anti-TNF-α treatments have shown the crucial role of this cytokine in the RA. Further studies are also needed to show benefits in the accelerated atherosclerosis in RA.

Rheumatology key messages

- RA accelerates atherosclerosis.
- Common inflammatory mediators are crucial in RA and atherosclerosis.
- Cytokines are the most promising targets to reduce atherosclerosis in RA.

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