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Platelet Function in Rheumatoid Arthritis: Arthritic and Cardiovascular Implications

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Abstract

Patients with rheumatoid arthritis (RA) are at high risk of cardiovascular events. Platelet biomarkers are involved in inflammation, atherosclerosis and thrombosis. Cardiovascular and RA-associated factors can alter the structure and function of platelets, starting from megakaryocytopoiesis. Reactive megakaryocytopoiesis increases circulating platelets count and triggers hyperactivity. Hyperactive platelets target synovial membranes with subsequent local rheumatoid inflammation. Hyperactive platelets interact with other cells, and target the vascular wall. Accumulating evidence suggests that disease modifying anti-rheumatic drugs (DMARD) decrease platelet activity.

Key words: Rheumatoid arthritis, Platelet function, Biomarkers, Inflammation, Cardiovascular risk.

INTRODUCTION

Rheumatoid Arthritis (RA) is the most common inflammatory polyarthritis. RA affects approximately 1% of the adult western populations [1] and is characterized by inflammation of varying intensity with progressive destruction of synovial joints and physical disability. It has become evident that patients with RA are burdened with excessive cardiovascular disease (CVD). Chronic inflammation is implicated in the high prevalence of CVD risk factors (e.g., hypertension, dyslipidemia, diabetes), premature atherosclerosis and altered coagulation [2]. Atherosclerotic CVD in RA is being increasingly recognized as an extra-articular manifestation and as a model for research, diagnosis and treatment of atherothrombosis [3].

Considerable evidence indicates that patients with RA are prone to premature ischaemic heart disease (IHD), myocardial infarction (MI), and heart failure. There are multiple pathways that link these conditions, one being thrombosis [4-6]. A large cross-sectional study, which compared the prevalence of CVD and cerebrovascular disease in 9093 RA and 2479 osteoarthritis (OA) patients in the USA, revealed that RA was associated with an increased lifetime risk for MI (odds ratio [OR] 1.28, 95% confidence interval [95% CI] 1.24, 1.33) and heart failure (OR 1.43, 95% CI 1.28, 1.59) [7]. The Rochester Epidemiology Project, a large population-based inception cohort, followed 603 RA patients for almost 40 years, and demonstrated that CVD, the leading cause of morbidity and mortality in RA, accounted for 49.7% of deaths [2]. Throughout follow-up, mortality rates were significantly higher in subjects with RA than without (39.0 vs. 29.2/1,000 person-years) [8]. It also revealed a striking difference in the cumulative incidence of heart failure between RA and non-RA populations at 30 years of follow-up (37.1% vs. 27.7%, $p < 0.001$), emphasizing how heart failure, a prothrombotic state [9-10], contributes to the excess mortality in RA.

The high prevalence of established CVD risk factors, such as smoking, hypertension, diabetes, only play a part in the increase in CVD [11]. Inflammatory mediators, endothelial dysfunction, and coagulation have also been extensively studied in RA [12].

Pathogenic factors that associate with active synovitis, bone and cartilage destruction in RA are present in high-grade inflammatory conditions and atherosclerosis [13-16]. Such factors may create an environment in which platelet activation amplifies CVD risk. A few of the known potential 'platelet agonists' include: oxidative stress, hyperinsulinemia, oxidized low density lipoprotein (oxLDL), C-reactive protein (CRP), tumor necrosis factor alpha (TNF α),

interleukins -1, -6, -18, RANK ligand, CD40 ligand, matrix metalloproteinases, monocyte chemotactic protein-1, fractalkine, and adipocytokines (Fig. 1) [2-5, 12-16].

In this review we discuss platelet function in RA and how this relates to CVD pathology.

SEARCH STRATEGY

The literature search was carried out in MEDLINE for English-language original research papers published from 1981 to 2009 using the following search terms related to the markers of platelet activation and RA: platelet function, atherothrombosis, cardiovascular disease, cardiovascular risk, rheumatoid arthritis, synovial fluid, rheumatoid synovium, mean platelet volume, P-selectin, platelet aggregation, platelet count, platelet microparticles, gene polymorphisms, CD40 signaling, platelet chemokines, disease modifying antirheumatic drugs. The reference lists of the selected articles were also hand searched to identify important and highly cited reviews on platelet function in inflammation.

PLATELET ACTIVATION WITHIN JOINTS IN RA

Though there is little evidence that platelets are directly involved in joint inflammation in RA, studies have shown an increased number of platelets and platelet-derived proteins (growth factors) within the synovium and synovial fluid [17-20]. High platelet counts in synovial fluid associate with rheumatoid factor (RF) and markers of synovial leukocyte activation in inflammatory arthritis, but not osteoarthritis [18, 21].

In RA, activated platelets, alone or together with other inflammatory cells and mediators, may play a significant role in thrombus formation, synovial microcirculation, and destruction of cartilage [22-25]. In a murine model of knee joint arthritis, platelets were found to interact with and adhere to endothelial cells (ECs) and leukocytes in the inflamed synovial vessels. Subsequent platelet aggregation leads to thrombus formation and alteration of the synovial microcirculation [22]. In a series of experiments in antigen-induced arthritis, P-selectin, an adhesion molecule produced by platelets and ECs, was shown to be crucial for the interaction of platelets, leukocytes, and ECs in the inflamed joints [23-24]. Other platelet-derived proteins also exhibited pro-inflammatory and joint destructive actions. In fact, platelet-derived growth factor, a potent angiogenic agent, was shown to induce synovitis and pannus-like hyperplasia in a rabbit model of RA [25].

Further evidence on platelet involvement in rheumatoid synovitis relates to the presence of platelet factor 4 in the synovial fluid, a chemokine and thrombotic agent with capacity to bind to antithrombin III and neutralize heparin [19]. Compared with circulating platelets, the surface of platelets in the synovial fluid contains higher levels of platelet factor 4; this suggests migration of circulating platelets and targeted action against rheumatoid joints [19]. Alternatively, constituents of rheumatoid synovial fluid may recruit platelets from the circulation and facilitate their prothrombotic and proinflammatory effects within the synovium [26-27]. Evidence suggests an association between platelet reactive IgG antibodies in the synovial fluid and heightened platelet activity [27].

To sum up, alpha granules and dense bodies of platelets, activated by systemic rheumatoid inflammation, may release their own inflammatory and immune mediators, facilitating initiation and propagation of synovitis. Though precise mechanisms of platelet activation are not fully understood, it is plausible that inhibition of platelets with subsequent decrease of platelet-derived inflammatory markers may have beneficial effect on the course of arthritis.

ACTIVATION OF CIRCULATING PLATELETS AND POTENTIAL IMPLICATIONS IN RA

Platelets are an important component of thrombogenesis and are involved in inflammation, endothelial dysfunction and atherogenesis [28].

A normal blood platelet count ranges from $150-400 \times 10^9/L$. A platelet's lifespan is 8-10 days, and normal daily release from the bone marrow is about 10^{11} (this may increase 10-fold in conditions of increased platelet turnover) [29]. Although megakaryocytes contain a nucleus and the whole biosynthetic apparatus, their offspring, newly formed 'reticulated platelets' are anucleate. However, for at least 24 hours after the release from the bone marrow, young platelets contain messenger RNA (mRNA), facilitating synthesis of platelet proteins [30]. An intensive stimulation of the bone marrow and increased platelet turnover may occur in response to an excessive production of inflammatory cytokines with the resultant increase of reticulated platelets. These platelets produce proteins, which lead to clot formation [31]. During activation, aggregation, adhesion to other cells and thrombotic plug formation platelets undergo shape and volume change. Aggregation is associated with the release of platelet-derived vasoactive and hemostatic substances [32].

Mean platelet volume (MPV)

The complexity of platelet physiology and its inter-relations with other biomarkers make it difficult to assess links between platelet activation, inflammation and atherogenesis. Even interpretation of changes in MPV [33] is not always straightforward in RA.

Availability of automated blood cell analyzers has made the measurement of platelet count and morphology common practice. MPV is emerging as an indicator of platelet reactivity, which could estimate cardiovascular risk [34]. In physiological conditions, an increased MPV reflects the predominance of young reticulated platelets in the circulation due to increased platelet turnover. MPV has been viewed as a reflection of activation when they transform from normal discoid to spheric shape with protrusion of pseudopodia and increase in the size [35-37]. Large platelets are associated with higher levels of IgG antibodies against platelet membrane glycoproteins IIb/IIIa, Ib and V [38] and an increased release of thromboxane A₂, beta-thromboglobulin and P-selectin [35,36,39]. Elevated MPV has been linked with heightened risk in patients with hypertension, obesity, diabetes, smoking, and hypercholesterolemia [40,41]. Furthermore, increased MPV is related to acute vascular events such as destabilization of atherosclerotic plaque, unstable angina, MI and paroxysmal atrial fibrillation [40,42]. MPV is an independent risk factor and predictor of MI in predisposed subjects [43,44]. In a large prospective cohort of patients with established cerebrovascular disease (n=3134), MPV predicted stroke within 4 years of follow-up with an 11% increased relative risk of stroke with each femtoliter increment of MPV [45]. A recent study identified significantly high MPV in patients with familial Mediterranean fever [46], suggesting a link between inflammation, platelet activation, and prothrombotic state.

MPV changes have been observed in some but not all studies in RA [47-48] (Table 1). In one study, MPV of 32 active RA patients was significantly lower than that of patients with osteoarthritis and healthy subjects. This finding was accompanied by increased disease activity, measured by Disease Activity Score 28 (DAS28), platelet count and biomarkers of inflammation, which suggested that platelet activation in RA is associated with reactive megakaryocytopoiesis [48] as part of active inflammation [49-50]. Small MPV may also reflect accelerated maturation and short lifespan of platelets in active RA. In contrast, in another prospective study [47], MPV significantly decreased alongside CRP, IL-6 and platelet count in response to a 2-year anti-rheumatic treatment, questioning the inverse correlation between MPV and thrombocytosis, observed by others in RA [48] and inflammatory bowel disease [51]. These results emphasize the need for larger studies to

clarify the discrepancies. An additional challenge relates to the methodological issues of MPV measurement, which is not a static variable and ranges widely with changing profiles of endogenous platelet agonists, treatment modalities, blood sampling, and storing.

Measuring platelet aggregation

A relatively simple test of platelet function is platelet aggregometry. This can be performed using platelet-rich plasma or whole blood and different platelet agonists (e.g., arachidonic acid, adenosine diphosphate [ADP], collagen, thrombin, epinephrine and modified immunoglobulins) [52]. The agonist is added to the suspension, and a dynamic measure of platelet clumping is recorded. Platelet aggregometry is widely considered as a ‘gold standard’ of platelet function assessment. As an in-vitro test, it has limitations (e.g., absence of interaction with other blood cells, artefacts occurring during sampling, centrifugation and platelets separation). Whole blood platelet count-based aggregometry overcomes some of these limitations [52].

In spite of the limitations, some studies have proven platelet aggregometry to be useful for detecting hyperactivity of platelets in RA [53] and assessing efficiency of anti-rheumatic drugs [54,55].

Platelet aggregometry studies identified triggers of excessive platelet aggregation. Examples are rheumatoid seropositivity, antibodies against beta-2-microglobulin and circulating immune complexes (another link between autoimmune reactions and prothrombotic state in RA) [53,56,57]. Interestingly, increased in-vitro platelet sensitivity to agonists and autoimmune factors has also been found in other rheumatic diseases (e.g., polymyalgia rheumatica, systemic sclerosis, gout), where common mechanisms of immune complex formation and accelerated atherogenesis can be inhibited by antiplatelets [58].

Thrombocytosis ($>400 \times 10^9/L$), characteristic for active RA, has been associated with increased sensitivity to platelet agonists, such as collagen and epinephrine, suggesting a pathological link between thrombocytosis and the arachidonic acid cascade [59]. A crucial role of arachidonic acid metabolites is also evident in the light of normal sensitivity to ADP, observed in platelet aggregation studies in RA [50].

Soluble P-selectin

The concentration of soluble P-selectin in plasma reflects in-vivo platelet activation [60-62]. Platelets, ECs and macrophages are all sources of this glycoprotein [63]. Its high plasma concentrations are largely associated with excessive release from alpha granules of platelets rather than from other sources [64,65]. Soluble P-selectin facilitates interaction of platelets with T-lymphocytes, neutrophils, monocytes and ECs at the sites of rheumatoid inflammation [23]. Cellular interactions require active participation of another adhesion molecule, P-selectin Glycoprotein Ligand-1 (PSGL-1), expressed on activated platelets, lymphocytes, monocytes and leukocytes [66]. By binding PSGL-1, P-selectin mediates adhesion of platelets and formation of complexes with leukocytes or monocytes. A further action of bound P-selectin is the up-regulation of other adhesion molecules, and the tethering and rolling of leukocytes into the endothelium [67]. In collagen-induced murine arthritis, disruption of P-selectin/PSGL-1 complex by PSGL-1 binding antibodies markedly suppressed leukocyte recruitment into the inflamed synovium and down-regulation of TNF synthesis by synoviocytes [68].

Not surprisingly, elevated levels of soluble P-selectin correlate with acute phase reactants and reflect the intensity of systemic inflammation in RA [60-62,69,70]. In fact, of a number of adhesion molecules, only P-selectin significantly correlates with RA activity [61]. Additionally, P-selectin associates with MPV [47] and platelet count [69], which implicates the role of circulating platelets in the elevation of P-selectin concentrations in RA. The role of P-selectin expression may vary widely, depending on the presence of articular and extra-articular rheumatoid manifestations. This is why in some observations soluble P-selectin levels were close to normal [71] or there were no associations with shifts of immune markers (e.g., soluble IL-2 receptor, a modulating protein of T-lymphocyte activity) [61].

Several studies have failed to provide evidence of direct involvement of soluble P-selectin in vasculitis, atherosclerotic disease or myocardial dysfunction in RA [33,62,70]. However, these studies had some limitations, such as inappropriate case-control design, small number of patients, lack of representative population of patients, neglect of confounding effects of acute-phase reactants interacting with P-selectin, or lack of correction required in the case of therapy with DMARDs and cardiovascular drugs, known to inhibit platelet function.

Flow cytometry analysis of platelet membrane-bound proteins

Flow cytometry has expanded the opportunities for comprehensive assessment of platelet activity [72-74]. Using specific monoclonal antibodies against different proteins of platelets and other cells, flow cytometry assesses markers of degranulation of alpha granules (CD62P), lysosomes, dense bodies (CD63) of platelets, conformational changes of platelet receptors GP IIb/IIIa and Ib, the presence of circulating complexes of platelets with leukocytes and monocytes and platelet-derived microparticles in autoimmune diseases [75]. Flow cytometry analysis is instrumental in monitoring long-term effects of drugs on platelet function [76]. The number of P-selectin (CD62P) positive cells detected by flow cytometry strongly correlates with RA activity and inflammatory markers [33,77]. This reciprocates the results of ELISA tests, implying overproduction of soluble P-selectin due to platelet involvement in rheumatoid inflammation.

Platelet-bound P-selectin overexpression has been associated with hypertension, diabetes and heart failure [78-80]. A retrospective cohort study with 517 subjects with diabetes, hypertension and hyperlipidemia revealed positive correlation of platelet-bound P-selectin with intimal-medial thickness (IMT), atherosclerotic plaques and stiffness of the carotid arteries [81]. Similar associations might be expected with cardiovascular co-morbidities in RA.

Platelet-derived microparticles (PDM)

P-selectin is intimately related to the functioning of PDM in RA [82]. PDM have been increasingly recognized as markers of platelet activation and potent prothrombotic agents. PDM increase in quantity and activity in conditions associated with oxidative stress, autoimmunity and thrombosis (e.g., antiphospholipid syndrome, acute coronary syndromes, venous thromboembolism, and sepsis) [83].

Flow cytometry can track the origin of microparticles. These are tiny vesicles (0.1-1 μm) with a complex array of proteins on the surface, which originate from platelets, ECs, monocytes, lymphocytes, and leukocytes. Sequestration of microparticles occurs due to platelet activation, aggregation, interaction with leukocytes, monocytes, Ecs, and spontaneously. High concentrations of PDM have been found in active RA independent of platelet count, suggesting a relation with activating factors other than thrombocytosis [82]. An interesting hypothesis was proposed that circulating PDM play a more important pathogenic role than the same PDM within the inflamed rheumatoid joints [84]. Actually,

PDM from rheumatoid platelets are predominantly found in the plasma, while more microparticles in the rheumatoid synovial fluid originate from leukocytes or monocytes [85]. It is likely that inflammatory and thrombogenic targets of PDM differ at various stages of RA (i.e., migration and rolling of circulating microparticles into the inflamed synovium at the initial active stage, and opposite migration toward systemic circulation with advancing joint inflammation). This might partly explain why despite a significant elevation and correlation of circulating PDM with DAS28 in some studies [82], normal levels of the same PDM are observed in others, where high levels of CD62P, platelet-monocyte complexes and soluble CD40L suggested active RA [33].

CD40 Ligand/CD40 complex

CD40 ligand (CD40L), a member of the TNF family, is expressed on the platelet membrane via arachidonic acid-mediated activation [85]. More than 90% of soluble CD40L is produced by activated platelets [85]. CD40L binds to platelet GPIIb/IIIa to stabilize arterial thrombus. Platelet-derived soluble CD40L also binds to its cognate receptor CD40 constitutively expressed on T-, B-lymphocytes and monocytes, thereby facilitating inflammation [66]. The CD40L/CD40 complex stimulates the release of chemokines, such as Regulated upon Activation, Normal T cell Expressed and Secreted (RANTES) and Monocyte Chemoattractant Protein-1 (MCP-1) from platelets and through this link reinforces T-lymphocyte-mediated immune reactions [86-88]. In experimental studies, CD40L/CD40 interaction was shown to up-regulate IgG RF production by B-lymphocytes, which was blocked by administration of CD40L neutralizing antibodies [89]. A strong correlation between soluble CD40L and IgM/IgG RF was also found in RA patients [90]. CD40L through the recruitment of leukocytes and other inflammatory cells to the sites of vascular injury may also be involved in endothelial dysfunction. Although increased levels of soluble CD40L were observed in active RA [33] and rheumatoid vasculitis [90], interactions of CD40L/CD40 with other markers of platelet activation, inflammation, autoimmunity, thrombosis and vascular risk in RA remain obscure.

GENETIC MARKERS OF PLATELET HYPERACTIVITY IN RA

Genetics may be relevant to platelet hyperactivity in RA. Platelet membrane GP IIIa polymorphism with antigen Ib positivity is a likely genetic factor to predispose to

atherothrombotic events. Positive Ib allele was found in one third of RA patients [91]. It is well known that the threshold of platelet activation in healthy persons and patients carrying Ib allele is significantly decreased. RA patients carrying this allele have significantly more circulating CD41a (GPIIb/IIIa) positive platelet aggregates and exhibit an enhanced platelet response to ADP, compared with patients without this allele. Moreover, the difference in response to different concentrations of ADP (0.5, 1 and 2 μ Mol) between Ib allele-carriers and non-carriers was confined to the 0.5 μ Mol concentration, which represents the ADP concentration inducing platelet aggregation not only through the activation of ADP receptors, but also via the arachidonic acid cascade. Thus, many RA patients, even those taking anti-platelet drugs, may exhibit platelet hyperactivity. The studies on GPIIIa polymorphism in RA may provide a useful tool for stratifying patients at high risk of cardiovascular events and selecting candidates for aggressive anti-platelet therapy.

Polymorphism of another protein from alpha granules of platelets, transforming growth factor-beta1 (TGF-beta1), also merits consideration. Many cells release TGF-beta1 into the circulation but platelets are the most important contributors. This cytokine possesses anti-inflammatory properties, preserving endothelial integrity, avoiding excessive destruction of the connective tissue and progression of atherosclerosis [92]. TGF-beta1 deficiency accelerates atherosclerosis. High concentrations of TGF-beta1 are observed in inflammatory and prothrombotic states and may, through negative feedback, suppress further activation of platelets. Recently, it was shown that platelet-derived TGF-beta1 enhances osteoclastic activity of the bone by activating Receptor Activator of NF-kappaB Ligand (RANKL), bridging platelet function with rheumatoid osteopathy [93].

The T-allele of T869C polymorphism is associated with reduced TGF-beta and high risk of MI in the general population. This single nucleotide polymorphism is also linked with the risk of RF positivity, RA development, joint damage, hypertension and mortality in RA [94]. Based on the obtained data, genetically determined platelet dysfunction is a probable pathophysiological link between rheumatoid and cardiovascular pathology.

ANTI-RHEUMATIC TREATMENT AND PLATELET FUNCTION

Methotrexate has proven efficacy to slow progression of RA, and it is largely recommended as the drug of choice for patients with early and advanced RA. In a case-control study of 613 RA patients with or without CVD, those who had ever received methotrexate had

significantly reduced risk of CVD (OR 0.11, 95%CI 0.02-0.56; $p < 0.05$). Similar trends were observed in those who had ever received sulfasalazine and/or hydroxychloroquine in combination with methotrexate [95]. In a landmark prospective study of 1240 RA patients, 191 fatal events were observed over 6 years of follow-up, of which 44% were due to CVD. Weighted Cox regression analysis revealed substantial benefits of low-dose methotrexate in terms of CVD mortality reduction (Hazard Ratio [HR] 0.3, 95%CI 0.2-0.7, compared with no methotrexate) [96]. This significant decrease of CVD mortality risk is not observed with other traditional DMARDs, such as sulfasalazine (HR 1.3, 95%CI 0.7-2.5 for those prescribed DMARDs other than methotrexate). The beneficial effect of methotrexate was independent of dose and folic acid supplementation. This study raises several questions: whether the reduction in CVD mortality is a result of a direct or indirect antithrombotic effect; and whether the same beneficial effect is achievable in patients with established CVD, in whom the risk of thrombotic events is greater.

The available data on the effect of methotrexate on thrombotic markers are inconclusive. In vitro, methotrexate suppresses expression of PSGL-1 by antigen-stimulated monocytes and through this may disrupt cellular interactions described within the frames of T-lymphocyte-mediated inflammation. However, in a clinical scenario, 6 weeks methotrexate therapy improved RA disease activity but failed to sufficiently suppress platelet hyperactivity, expressed by soluble levels of P-selectin, beta-thromboglobulin, platelet-leukocyte complexes, glycoprotein IIb/IIIa conformational change and binding of PAC-1 monoclonal antibody to platelets [72]. It was also shown that the plasma of RA patients receiving methotrexate, but not methotrexate plus diclofenac, induced significantly higher in vitro platelet aggregation than that of healthy controls ($p < 0.05$) [97].

Over the past decade, anti-TNF-alpha therapies (etanercept, infliximab and adalimumab) have proven to be pivotal in the treatment of RA. The effects of these drugs are mediated via reduction of chemokines (e.g., RANTES, MCP-1). Successful down-regulation of rheumatoid inflammation with anti-TNF-alpha agents is hoped to reduce associated CVD and thrombotic events.

A few short-term studies with infliximab in RA have revealed alterations in prothrombin fragment 1+2 and D-dimer [98], platelet count, adhesion molecules [99], with the most significant effect on soluble P-selectin; this suggests a reduction of thrombotic risk. Nevertheless, the benefits of anti-TNF-alpha treatment should be weighted against possible progression of heart failure in some patients, particularly those with CVD and advanced

heart failure. It is speculated that the suppression of TNF-alpha may interfere with homeostasis and may up-regulate anticardiolipin and other autoantibodies, leading toward platelet hyperactivity and thrombosis. Interestingly, a recent study with RA patients treated with infliximab over a period of 30 weeks proved that clinical non-responsiveness to infliximab was associated with high levels of platelet factor 4 [100]. Finally, the risk of adverse thrombotic effects of TNF-alpha blockade may further increase in those with high disease activity, taking high dose glucocorticoids, cyclooxygenase-2 (COX-2) inhibitors (coxibs) and heart failure medications.

Platelet effects have also been studied with other DMARDs. Sulfasalazine significantly reduced platelet count [101] and soluble P-selectin [102]. Hydroxychloroquine therapy is believed to suppress GPIIb/IIIa, GPIIIa platelet receptors and to decrease platelet count in RA [103].

Despite some adverse effects of glucocorticoids on CVD risk factors in RA (e.g., insulin resistance, obesity, hypertension), these drugs are still viewed as essential for anti-rheumatoid therapy. Through the suppression of inflammation steroids may also suppress platelets and, thus, reduce CVD. It is likely that adverse cardiovascular effects of steroids are dose- and time-dependent [104].

The arachidonic acid cascade plays a key role in platelet activation, which favours therapeutic potential of NSAIDs. Of these, non-selective NSAIDs (naproxen) but not selective COX-2 inhibitors (meloxicam) have both anti-inflammatory and anti-platelet effects [55]. Indomethacin was shown to suppress platelet activation-dependant osteoclastopoiesis, similarly to the action of osteoprotegerin, a natural decoy receptor of RANKL [105].

CONCLUDING REMARKS

It is now recognized that RA patients are prone to accelerated atherosclerosis and premature CVD. It is also obvious that established CVD risk factors alone do not fully account for increased cardiovascular mortality in these patients.

Evidence suggests that platelets are involved in inflammation, endothelial dysfunction and thrombosis, and are potential targets for anti-rheumatoid and cardiovascular therapy in RA (Fig. 2). Systemic rheumatoid inflammation mediated by numerous primary (IL-1) and secondary cytokines (TNF-alpha, IL-6, IL-8), growth factors, and autoantibodies stimulate

platelet turnover in the bone marrow in RA. As a product of megakaryocytopoiesis, platelets are anucleate cells with a lifespan of 8-10 days. Within this relatively short period, especially over the first 24 hours, platelets can synthesize proteins on their mRNA and can produce microparticles. Platelets may exceed leukocytes, monocytes and other cells in the production of P-selectin, CD40L, platelet-derived growth factor, and, thereby, can take a leading position in the process of systemic rheumatoid inflammation. Platelets are also known to produce large amounts of TGF-beta1, which suppresses excessive platelet activation and destruction of the connective tissue, but may fail to exert its beneficial action in RA due to several reasons, genetic polymorphisms being the most probable.

Platelets, platelet factor 4, platelet-derived growth factors, serotonin and microparticles have been found in the synovial fluid of patients with RA, where these agents may disturb microcirculation and fuel synovitis. However, it is highly likely that circulating platelets, including those originating from the synovial fluid, possess more important vasculopathic function. The latter merits consideration in specifically designed prospective studies on cardiovascular risk in RA.

Recent attempts to associate a single marker of platelet function (e.g., P-selectin, MPV) with accelerated atherosclerosis and CVD in RA have failed [70]. It is, therefore, important to further investigate shifts of several markers of platelet function in response to DMARDs and anti-platelets in RA.

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Table 1. Studies on circulating platelets in RA.

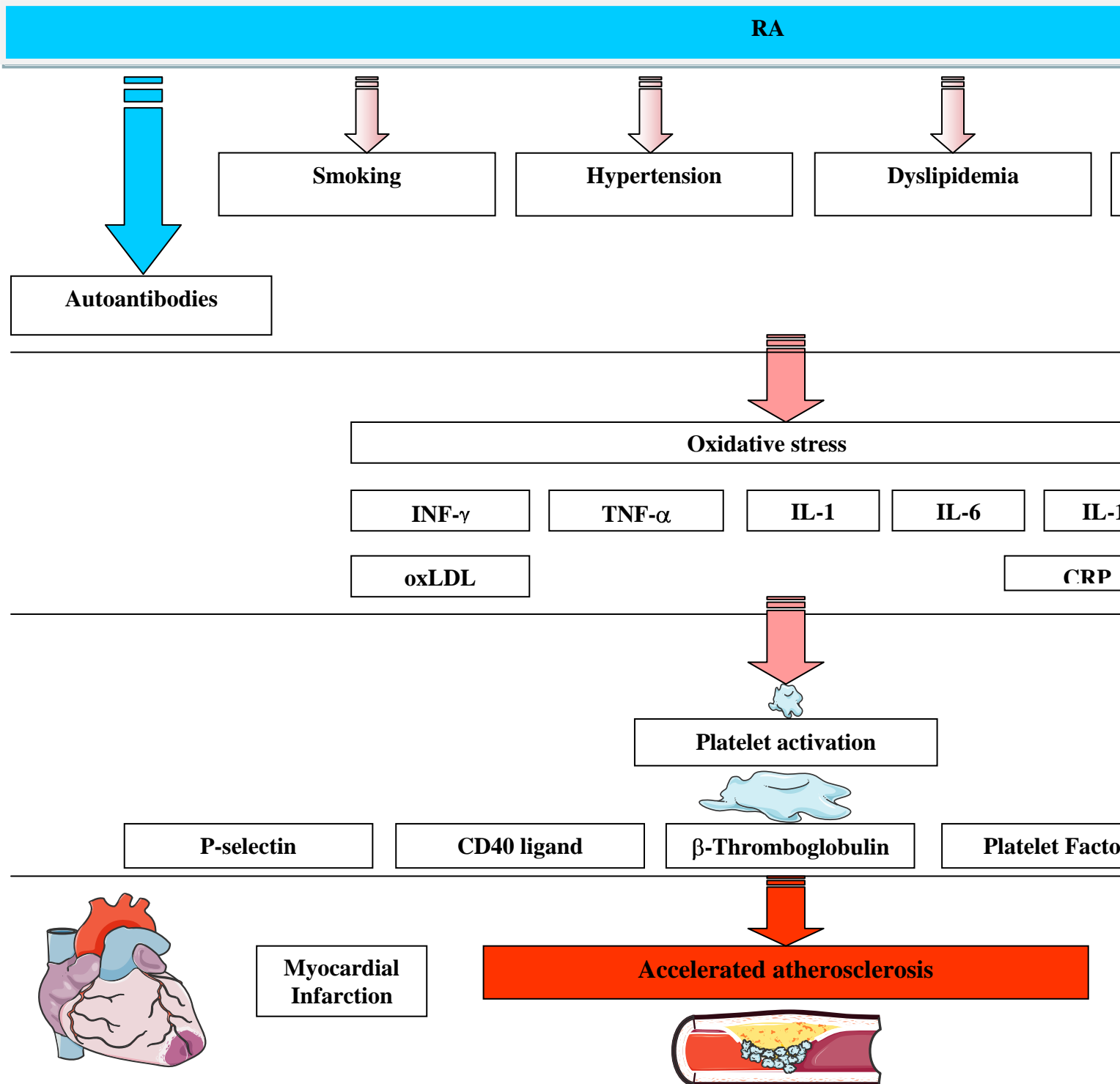
Study	N	Design	Tests	
[47]	16	Assessments at active stage and after 2 years of treatment (steroids, methotrexate, NSAIDs). Healthy controls were not recruited	MPV, platelet count, thrombopoietin, P-selectin	Mean MPV decreased, platelet count, C-reactive protein did not change. MPV increased at active stage
[48]	32	Assessments at active stage and after 2 months of treatment (not specified). RA patients were matched with osteoarthritis patients and healthy subjects	MPV, platelet count	Baseline MPV decreased with disease and healthy controls respectively; p=0.001. At follow-up, RA patients had higher platelet count, C-reactive protein
[49]	39	Cross-sectional study	P-selectin, platelet count	P-selectin was higher in RA patients with thrombocytosis. P-selectin and C-reactive protein were correlated with symptoms
[60]	22	Cross-sectional study	P-selectin	Significant elevation of P-selectin, ICAM-3, VCAM-1. P-selectin correlated with disease activity
[61]	25	Cross-sectional study with RA, SLE, SS and healthy control	P-selectin	P-selectin was higher in RA patients

		groups		differing from t interleukin-2 re SLE patients
[62]	31	RA and osteoarthritis patients were compared. Treatment in RA group with methotrexate, sulfasalazine, corticosteroids, leflunomide, etanercept, chloroquine, NSAIDs	P-selectin, cutaneous capillary blood flow velocity	Majority of RA There was no d osteoarthritis. P flow correlated correlation with
[70]	153	Cross-sectional study with healthy controls and 3 subgroups of RA patients treated with DMARDs, NSAIDs, ACE-I/ARB, beta-blockers, aspirin: with or without cardiovascular risk factors, and with left ventricular systolic dysfunction	P-selectin	Compared with RA group (p<0 selectin levels b because of the r production
[33]	27	RA patients with and without disease activity, compared with healthy controls. Treatment with methotrexate, sulfasalazine, steroids, leflunomide, etanercept, chloroquine, NSAIDs, COX-2 inhibitors, aspirin	CD62P, microparticles, platelet complexes with monocytes and neutrophils, soluble CD40L	P-selectin, com not platelet mic was no correlat and between pl intima-media th
[77]	28	Comparison between RA patients with and without disease activity, and healthy controls	CD62P and CD63	Expression of C RA group, whe CRP, ESR
[82]	19	Comparison between RA patients with and without disease activity, and healthy controls. Patients were treated with methotrexate, sulfasalazine, hydroxychloroquine and NSAIDs	Platelet count, PMP	Significantly in RA patients. PM
[90]	39	RA patients (9 with vasculitis) were compared with healthy subjects	Soluble CD40L	sCD40L was hi those with vasc correlated (r=0.

Abbreviations. RA: rheumatoid arthritis; NSAIDs: non-steroidal anti-inflammatory drugs; MPV: mean platelet volume; CRP: C reactive protein; IL-6: interleukin-6; ESR: erythrocyte sedimentation rate; DAS28: disease activity score 28; ICAM-1: intercellular adhesion molecule-1; ICAM-3: intercellular adhesion molecule-3; VCAM-1: vascular cell adhesion molecule-1; SLE: systemic lupus erythematosus; SS: systemic sclerosis; CD62P: platelet membrane-bound P-selectin; CD40L: CD40 ligand; sCD40L: soluble CD40L; PMP: platelet microparticles; IgM: immunoglobulin M; IgG: immunoglobulin G; RF: rheumatoid factor.

Figure 1. Possible factors involved in platelet activation and atherogenesis in RA.

Abbreviations. RA=rheumatoid arthritis; INF- γ =interferon-gamma; TNF- α =tumor necrosis factor alpha; IL-1=interleukin-1; IL-6=interleukin-6; IL-18= interleukin-18; oxLDL= oxidized low density lipoprotein; CRP=C-reactive protein



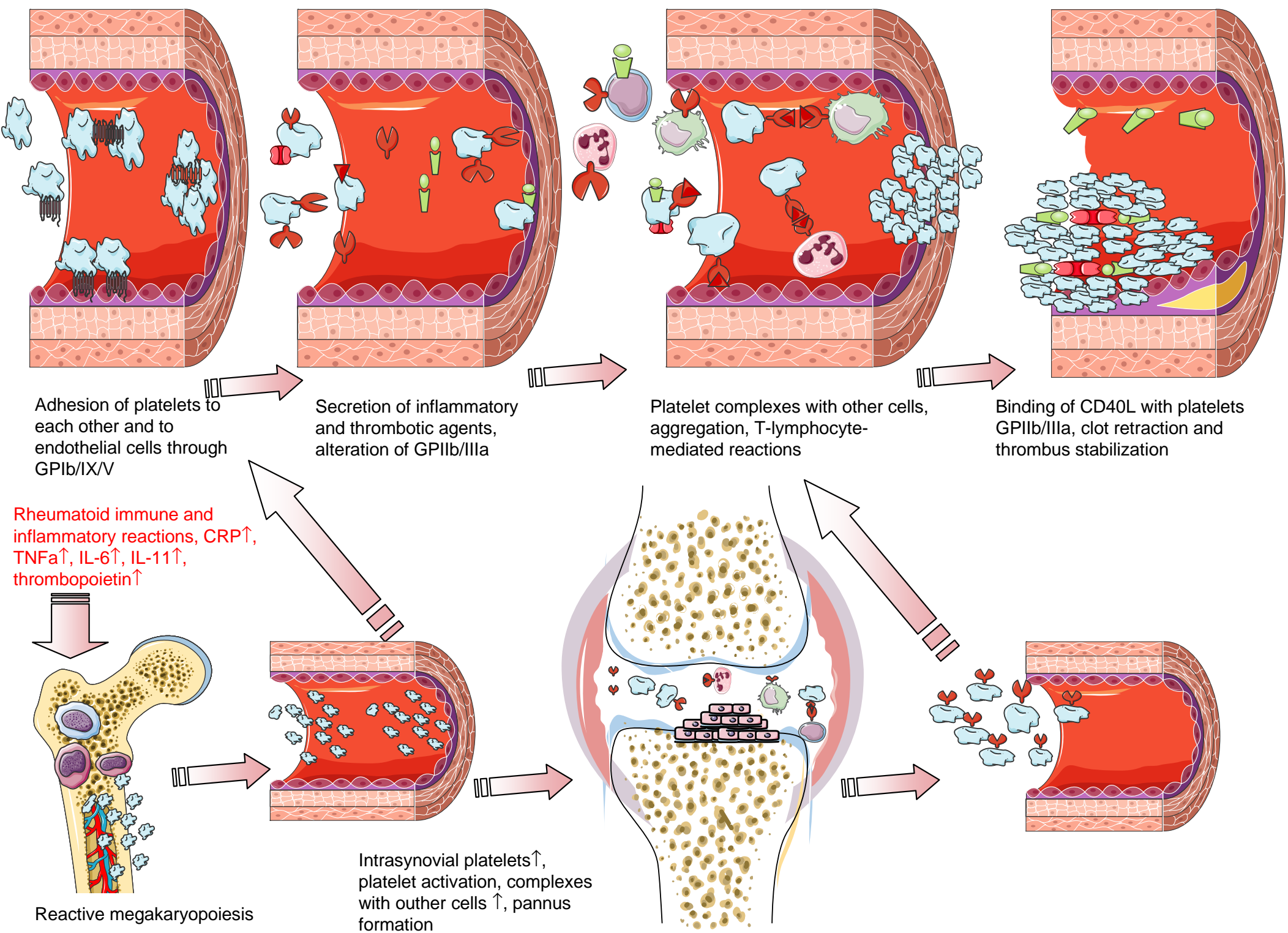
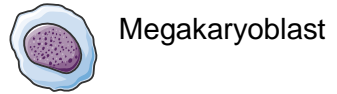


Figure 2. The role of circulating and synovial rheumatoid platelets



Megakaryoblast



Megakaryocyte



Platelet



Monocyte



T-lymphocyte



Leukocyte



GPIb/IX/V



GPIIb/IIIa



P-selectin



PSGL-1



CD40L