A Platelet’s Guide to Synovitis

Giacomo Cafaro MD, Elena Bartoloni MD, Alessia Alunno MD PhD, Onelia Bistoni BSc, Sabrina Cipriani PhD, Fabiana Topini PhD and Roberto Gerli MD

Rheumatology Unit, Department of Medicine, University of Perugia, Perugia, Italy

Platelets have the ability to influence the immune system and the inflammatory process and may be strongly involved in the whole pathogenic process of chronic inflammatory joint diseases, such as rheumatoid arthritis. They may play a significant role even before the clinical onset of the disease, contributing to the loss of tolerance of the immune system and the induction of autoimmunity. Subsequently, they can interact with the most important cellular players involved in autoimmunity and inflammation, namely innate immunity cells and T cells, and may eventually contribute to the building of inflammation in the synovium, thus inducing the activation, migration, and proliferation of fibroblasts that eventually lead to joint damage. Due to their peculiar features, studying the behavior of platelets is a challenging task; however, platelets may prove to be valuable therapeutic targets in the future.

Platelets play an important role in the immune system and are involved in numerous autoimmune conditions, including inflammatory joint diseases

Potential involvement in the pathogenesis of autoimmune and inflammatory diseases [1].

In this short review, we present the most important available data on the role of platelets toward the development of synovitis, one of the main features of inflammatory joint diseases such as rheumatoid arthritis (RA). RA is considered the prototype of autoimmune inflammatory joint diseases and is characterized by a process that starts from the production of autoantibodies and ends with irreversible joint damage and disability [2].

Autoantibodies and Platelets

One of the earliest steps in RA pathogenesis is the production of autoantibodies, the most well-known of which are rheumatoid factor (RF), anti-citrullinated peptides antibodies (ACPA), and anti-carbamylated peptides antibodies. Circulating autoantibodies have been shown to be present numerous years before the onset of the disease, suggesting a step-by-step process triggered by largely unknown factors.

Among their membrane receptors, megakaryocytes express class I major histocompatibility complex (MHC-I) and are able to endocytose peptides, process them, and load them on MHC-I molecules, essentially functioning as antigen-presenting cells (APC). Even more interesting, antigen-MHC-I complexes are transferred to newly generated platelets, which enter the bloodstream and can therefore spread and present the antigens to immune cells. In particular, activation of CD8+ T-cells by platelets has been demonstrated; however, it is plausible that the effect may be exerted on other immune cells [3]. Although not yet demonstrated, it is also possible that through this mechanism, platelets might be able to present autoantigens such as citrullinated proteins, thus inducing an immune response by T-cells.

One of the most important steps that triggers platelet activation and thrombus formation is the binding of the surface integrin glycoprotein (gp)IIb/IIa by components of the extracellular matrix, such as collagen and fibronectin. The binding of gpIIb/IIa by ECM components can in turn induce phosphorylation of the IgG Fc receptor IIa (FcγRIIa), thus allowing immune-complex-mediated platelet activation, another important mechanism by which platelets are involved in autoimmunity [4,5]. In addition, it is important to remember that fibronectin and
collagen are among the proteins that undergo citrullination and therefore may contribute to RA pathogenesis. It is reasonable to hypothesize that citrullinated collagen and ECM peptides are able to induce platelet activation (through gpIIb/IIIa but also through the integrin α2β1 and glycoprotein VI–GPVI) [6,7], and eventually trigger the secretion of α-granules that contain, among other substances, the soluble form of CD40L (sCD40L). sCD40L can bind CD40 on B-cells which, in turn, can produce RF [8], supporting the development of RA.

Another important marker of platelet activation is the secretion of platelet microparticles (PMP). PMPs are small vesicles (0.1–1 μm) actively generated by platelets on activation, which virtually contain all the components of the generating platelets, including organelles, nucleic acids, membrane phospholipids, and receptors. Citrullinated peptides have been found on their surface, suggesting their role as a source of autoantigens [9].

Further complicating the picture, ACPAs can activate platelets through FcyRIIa. As a consequence of this interaction, platelets shed the soluble form of GPV1 (sGPV1), which is found at higher concentrations in seropositive RA patients, compared to seronegative ones [7]. This phenomenon, together with the possible contribution of platelets in the generation of ACPAs, may represent an interesting immunologic feed-back/feed-forward mechanism that is worth further attention.

**HOMING OF IMMUNE CELLS TO THE SITE OF INFLAMMATION**

Following the onset of autoimmunity, independent of its initial development in the joints or not [10], there is little doubt that the main site of inflammation in RA is the synovium. For the inflammatory process to develop, a series of mechanisms take place that eventually induce the numerous cellular players involved in RA to migrate to the joints. There is a constantly growing body of evidence supporting a key role of platelets in this process. It is well-established that platelets promote the expression of adhesion molecules by endothelial cells and can themselves bind and promote adhesion and migration of leukocytes [11].

Moreover, platelets are among the most important producers of the chemokines CXCL7, CXCL4 (also known as platelet factor 4 - PF4), and CCL5 (also known as RANTES). CXCL7 and CXCL4 are strong inducers of neutrophil migration, although their functioning is complex. CXCL7 needs to be activated molecules by endothelial cells and can themselves bind and promote adhesion and migration of leukocytes [11].

Platelets can function as antigen-presenting cells, bind leukocytes, regulate their activation status, promote migration toward sites of inflammation, and stimulate proliferation and migration of synoviocytes deficient, when the signal transduction of PSGL-1 is impaired, or if PLTs are depleted from the circulation. These conditions emphasize that PLTs, through the interaction of P-selectin with its receptor on neutrophils, are essential to orchestrate leukocyte migration, along with the production of neutrophil extracellular traps (NET) [14].

To attract cells to the site of inflammation, an increase in vascular permeability is required. It is an early step in every inflammatory process and the presence of endothelial fenestrations in inflamed synovium has been demonstrated. Theoretically, the formation of endothelial gaps exposes ECM components to the vascular lumen, which should trigger the activation of platelets to preserve vascular integrity. However, this phenomenon is strongly inhibited during inflammation. When the K/BxN murine model of RA is depleted of platelets, the formation of endothelial gaps is completely inhibited, thereby suggesting that not only their physicologic repairing response is inhibited, but that they may actively contribute to the increase of synovial vascular permeability. This event is likely mediated by the release of serotonin (5HT) by platelets on binding of collagen to GPV1 receptor. By preventing the accumulation of 5HT in platelets, which do not produce 5HT but uptake it in large amounts from the circulation and store it in dense granules, serotonin reuptake inhibitors may exert an anti-inflammatory effect [15].

Although the endothelial fenestrations present in murine models have been shown to be large enough to allow the passage of PMPs (but not of platelets, which seem to preferentially migrate bound on the surface of neutrophils, independently
from the presence of endothelial gaps). PMPs are more abundant in RA synovial fluid (SF) compared to the circulation by several orders of magnitude, supporting the idea that local generation prevails on migration [9,15,16]. In addition, the characteristics of circulating PMPs in terms of dimensions, CD41 expression, and mitochondrial content is variable in RA patients, suggesting that the weight of platelets contribution to the pathogenesis of RA may not be equal among subjects [17].

PLATELETS AND INNATE IMMUNITY

PLTs express numerous receptors typical of cells involved in the innate immune response, such as toll-like receptors (TLR), complement receptors, and immunoglobulin receptors.

TLRs can bind various microbial (e.g., bacterial lipopolysaccharide) and endogenous ligands. The latter are known as damage associated molecular patterns and include alarmins, fibrinogen, and ECM components, which, in certain circumstances, are able to trigger an inflammatory response [18]. Although the presence of TLRs has been demonstrated on platelets, very little is known on their non-hemostatic functions. Of note, inhibition of TLR9 in murine models of arthritis reduces the aggressiveness of the disease [19]. Moreover, several endogenous ligands of TLRs are abundantly present in RA SF and citrullination of fibrinogen is probably an important phenomenon in RA pathogenesis [20]. Altogether, these observations suggest a potential significant role of platelet-TLRs that is worth further research. In addition, the downstream signaling of TLRs causes the release of inflammatory cytokines by immune cells, including IL-1α and IL-1β, which are also abundantly stored in platelets [21] and whose secretion may follow a similar pattern.

The relationship between platelets and neutrophils is a fundamental event in inflammation. The binding is mediated by P-selectin and its receptor PSGL-1, but also CD40 and CCL5. This binding also triggers a wide range of phenomena [22], such as NET formation, also known as NETosis. NETs are extracellular structures actively secreted by neutrophils on activation, which contain various components, including chromatin and proteases, whose main function is to engulf pathogens and promote their killing. Nonetheless, NETs are thought to be involved in RA pathogenesis as well. NET release by neutrophils is stimulated by the binding of platelets via not fully understood mechanisms, probably involving TLRs signaling. Platelets can also promote neutrophil survival by secreting growth factors and inflammatory cytokines, including IL-1β. Of interest, NETs can stimulate platelets to initiate thrombosis via binding of complement receptors, representing a potential mechanism underlying the increased cardiovascular risk observed in RA patients [21].

IL-1α and β can be de novo synthesized by platelets from stored mRNA and eventually secreted following activation. Data from a murine model of arthritis have shown that megakaryocytes can directly produce IL-1, making platelets a dispensable source, thus suggesting a potential role of megakaryocytes in the pathogenesis of arthritis that goes beyond the production of platelets [23].

As mentioned earlier, platelets can produce PMPs on activation. One of the peculiarities of PMPs is a significant exposure of phosphatidylserine on the outer phospholipid layer, similar to apoptotic bodies. This feature is at the basis of the ability of PMPs to bind leukocytes and to be phagocytosed. Neutrophils not only bind platelets but also PMPs, which have also been found in their cytoplasm, demonstrating the ability of leukocytes to endocytose them [9]. Membrane phospholipids also represent the substrate for thromboxane, prostaglandin, and leukotriene synthesis by immune cells, including platelets, which contain the enzyme thromboxane synthase A2, essential for hemostasis [24,25]. PMPs are internalized by other cells and the consequences are not clearly understood; however, the transfer of their mRNA, miRNA, ncRNA, mitochondria, and membrane phospholipid content to neutrophils may represent one of the mechanisms by which platelets and leukocytes regulate the functions of each other [9].

However, PMPs are not passively internalized by neutrophils. This process has been demonstrated to be tightly regulated by at least two enzymes, namely secreted phospholipase A2 group IIA (sPLA2-IIA) and 12-lypoxgenase (12-LO). sPLA2-IIA is present in great amounts in RA SF and is expressed by numerous cells. Its synthesis can be stimulated by IL-1α and IL-1β. Rather, 12-LO is mainly expressed by platelets and PMPs. sPLA2-IIA hydrolyses the second fatty acid tail of membrane phospholipids, releasing arachidonic acid (AA). Consequently, 12-LO catalyzes the conversion of AA into 12-hydroxyeicosatetraenoic acid (12-HETE), which binds the leukotriene B4 receptors 1 and 2 (LTB4 and LTB5) expressed by neutrophils, thus promoting PMPs internalization. Of note, only the S stereoisomer of 12-HETE is biologically active in this process [25]. Of interest, Porphyromonas gingivalis, a bacterium present in the oral cavity of healthy individuals and associated with periodontitis and a potential contributor of peptide citrullination due to its ability to express the enzyme peptidylarginine deiminase, can also stimulate the production of the enzyme sPLA2-IIA [10,25].

PLATELETS AND ACQUIRED IMMUNITY

The interactions between platelets and acquired immunity cells in the context of autoimmune diseases have not been extensively explored, although they represent a very interesting area of research, especially considering the pivotal role of lymphocytes in the pathogenesis of autoimmune and inflammatory joint diseases.

As mentioned previously, platelets can act as APCs, thus inducing the activation of acquired effector cells, such as T

There is growing evidence that platelets can modulate the differentiation of T helper cells into their sub-populations
Platelets were then able to bind circulating CD8+ cells, thus endothelial cells expressing hyaluronic acid residues via CD44. Cells homing toward liver cells by adhering to hepatic sinusoid inflammatory joint diseases that mainly target (directly or indirectly) platelet-lymphocyte ratio, and inhibition of platelet-cell contact dependent on multiple variables including time of incubation, thus, making interpretation of the data difficult. The published data we recently reviewed [24] suggest that platelets may exert both pro-inflammatory (promoting differentiation toward Th1 and Th17 phenotypes) and anti-inflammatory effects (supporting Treg differentiation and survival) on Th cells, which are dependent on multiple variables including time of incubation, platelet-lymphocyte ratio, and inhibition of platelet-cell contact [30-32]. Nonetheless, this aspect is worth careful attention, especially in light of the currently available treatments for inflammatory joint disease. The final contributors to joint disease in inflammatory articular diseases and mainly responsible for joint damage are synovial stromal cells, mainly fibroblast-like synoviocytes (FLS). For this reason, their role in both the onset and progression of arthritis has been a constant focus of research, including their relationship with platelets. It is well-known that platelets can respond to collagen as a physiological process in hemostasis. In fact, collagen and collagen degradation products, which are present at higher concentrations in the SF of RA patients, can bind multiple platelet receptors, including integrin α2β1 and GPVI, triggering their activation, thus inducing the production of PMPs which, by releasing IL-1α and IL-1β, can induce FLS to secrete great amounts of IL-6 and IL-8, well-known contributors to synovitis [16]. The interactions between platelets and FLS, however, can also be contact-mediated. In addition to lymphoid organs, the expression of podoplanin has been demonstrated on RA FLS, but not in healthy synovium. These observations have suggested a potential pathogenic role of such receptors, which as far as we know, lack an intracellular domain, rendering it unable to transduce the signal. Therefore, the effect of podoplanin can be exerted exclusively through its ligand CLEC1B mainly expressed by platelets [29]. When binding to podoplanin, CLEC1B transduces signals via phosphorylation of immunoreceptor tyrosine-based activation motifs, which in turn, activate spleen tyrosine kinase and eventually Bruton’s tyrosine kinase (BTK). This action results in platelet activation, including production of PMPs and secretion of IL-1, which have a pro-inflammatory paracrine effect on FLS. The same signal transduction cascade is triggered by the activation of GPVI [33]. The inhibition of BTKs for the treatment of RA is currently under investigation, especially considering the availability of commercial BTK inhibitors, which are currently used for malignant lymphoproliferative diseases [34]. However, the focus of current research is mainly on the role of BTKs on B-cell activity rather than on platelets, which to the best of our knowledge, has only been proven in murine models [33].

**Platelet functions are very complex and difficult to dissect, although in the future they may prove to be valuable targets for the treatment of autoimmune diseases**

**Platelets and Synoviocytes**

The final contributors to joint disease in inflammatory articular diseases and mainly responsible for joint damage are synovial stromal cells, mainly fibroblast-like synoviocytes (FLS). For this reason, their role in both the onset and progression of arthritis has been a constant focus of research, including their relationship with platelets. It is well-known that platelets can respond to collagen as a physiological process in hemostasis. In fact, collagen and collagen degradation products, which are present at higher concentrations in the SF of RA patients, can bind multiple platelet receptors, including integrin α2β1 and GPVI, triggering their activation, thus inducing the production of PMPs which, by releasing IL-1α and IL-1β, can induce FLS to secrete great amounts of IL-6 and IL-8, well-known contributors to synovitis [16]. The interactions between platelets and FLS, however, can also be contact-mediated. In addition to lymphoid organs, the expression of podoplanin has been demonstrated on RA FLS, but not in healthy synovium. These observations have suggested a potential pathogenic role of such receptors, which as far as we know, lack an intracellular domain, rendering it unable to transduce the signal. Therefore, the effect of podoplanin can be exerted exclusively through its ligand CLEC1B mainly expressed by platelets [29]. When binding to podoplanin, CLEC1B transduces signals via phosphorylation of immunoreceptor tyrosine-based activation motifs, which in turn, activate spleen tyrosine kinase and eventually Bruton’s tyrosine kinase (BTK). This action results in platelet activation, including production of PMPs and secretion of IL-1, which have a pro-inflammatory paracrine effect on FLS. The same signal transduction cascade is triggered by the activation of GPVI [33]. The inhibition of BTKs for the treatment of RA is currently under investigation, especially considering the availability of commercial BTK inhibitors, which are currently used for malignant lymphoproliferative diseases [34]. However, the focus of current research is mainly on the role of BTKs on B-cell activity rather than on platelets, which to the best of our knowledge, has only been proven in murine models [33].

**Joint Damage**

The end stage of inflammatory joint disease is damage accrual, which represents the principal cause of disability in patients. In fact, the final purpose of disease treatment is indeed the prevention and minimization of irreversible damage. The mechanisms that link inflammation to damage are multiple, all culminating in destruction of cartilage, tendons, ligaments, and bone via the degradation of ECM, induction of cell apoptosis, and bone resorption. The main players of this process are FLSs responsible for pannus formation and secretion of proteases, which

457
are significantly increased in the SF of patients with RA [36]. Although there is not much data on the role of platelets in this last phase of the process, some in vitro studies have shown that they can variably influence the proliferation and migration of FLSs, both essential aspects in the process of pannus formation and invasion. The ability of FLSs to migrate is well-known, both locally and through the blood stream. In fact, this ability has also been suggested as one of the mechanisms underlying the spreading of inflammation to multiple joints in RA [37]. In vitro experiments performed on RA FLS have shown that their ability to migrate through a transwell system and to invade the ECM-like matrix is significantly increased when they are co-cultured with platelet-rich-plasma (PRP), which is plasma with twofold to sevenfold higher concentration of platelets compared to circulation.

In addition, following co-culture with PRP, FLS synthesize more matrix metalloproteinase (MMP)-1, known to contribute in the degradation of cartilage [38]. These data are in accordance with our experiments that show an increased secretion of MMP-2 by RA-FLS following stimulation with platelets (unpublished data), which is also evident in OA-FLS [39].

Moreover, it is plausible that platelets may modulate osteoclastogenesis in RA, a mechanism at the basis of bone erosions, although very little data are currently available [19]. Due to the putative pro-proliferative effect of platelets on fibroblasts, and to their content in growth factors, treatment with PRP is currently used in the treatment of skin lesions and osteoarthritis, to improve symptoms, wound healing, and joint repair. There is some evidence that PRP is actually able to promote osteochondral damage repair, perhaps synergistically with synovial mesenchymal stem cells [40]. Although these apparently opposite effects may seem in contrast, they are not surprising. The behavior of platelets is very complex and indeed they are very easily influenced by environment and experimental conditions [24].

CONCLUSIONS

Since the first evidence of a potential involvement of platelets in the pathogenesis of RA, growing scientific interest has focused on the non-hemostatic functions of platelets, especially in the field of autoimmunity. As a consequence, a significant amount of data are currently available, although an accurate overall picture of the immune functions of platelets is still lacking. The reasons are manifold, including the complexity of platelet biology, the difficulty of handling them in vitro due to their strong predisposition to respond to external stimuli and the variability of their activation status which hamper the reproducibility of experiments.

However, there is enough evidence to be confident that platelets may have a role in virtually all pathogenic steps that lead to synovitis, although most of the data derive from murine models of RA. It will be very interesting to further dissect these phenomena in other inflammatory and autoimmune conditions.

The scientific community should begin to carefully look at platelets not as hemostatic particles with ancillary immune functions but as actual players of the immune system. This point of view is indispensable in order to better understand the biology of platelets and to recognize them as potential therapeutic targets to treat inflammatory and autoimmune diseases.

Correspondence

Dr. R. Gerli
Rheumatology Unit, Department of Medicine, University of Perugia, Piazzale Menghini 1, 06129, Perugia, Italy
Phone: (39-075) 578-3975, (39-075) 578-3436
Fax: (39-075) 578-3444
email: roberto.gerli@unipg.it

References


