A Platelet's Guide to Synovitis

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ABSTRACT: 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.

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As more evidence accumulates, the involvement of platelets in the immune system is attracting growing attention by the scientific community. Platelets are circulating cellular fragments produced in the bone marrow by megakaryocytes. They are anucleate and thus incapable of nucleic acid transcription; however, they retain the cytoplasmic organelles of their parent cells, including endoplasmic reticulum, Golgi apparatus, mitochondria, and intracellular and membrane receptors. Platelets also maintain a significant amount of messenger RNA (mRNA), microRNAs (miRNA), and non-coding RNAs (ncRNA). Due to these features, platelets are able to dynamically respond to external stimuli by controlling mRNA translation, performing protein synthesis and secreting peptides in the surrounding environment. These characteristics are fundamental within the complex hemostatic response. However, evidence is accumulating that a significant proportion of platelet functions and characteristics are redundant in terms of hemostasis, further supporting a physiological role within the immune system, but also a

> **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/IIIa by components of the extracellular matrix, such as collagen and fibronectin. The binding of gpIIb/IIIa 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 FcγRIIa. As a consequence of this interaction, platelets shed the soluble form of GPVI (sGPVI), 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 by neutrophils themselves to be chemotactically active, while CXCL4 can antagonize CXCL7 at low concentrations, as it synergizes with it when present at high concentrations. In addition, CCL5 is involved in the recruitment of monocytes, whose survival can be promoted by CXCL4 [12]. However, a recent study has shown that CXCL4 and CXCL7 are not PLT-specific chemokines, as previously thought. They are significantly expressed by RA-synovial macrophages as well, while more abundantly in

the very early stages of disease compared to established disease [13]. Although it is unknown whether this observation may change the paradigm of platelet-derived CXCL4 and CXCL7, macrophages can probably induce neutrophil migration as well.

Another interesting observation comes from a study that analyzed the behavior of neutrophils during the various phases of extravasation (adhesion to the endothelium, rolling, and crawling) by intravital microscopy following treatment of mice with tumor necrosis factor-alpha (TNF-α). To allow cells to move in the surrounding environment, a dramatic change in their intracellular and membrane organization takes place, leading to a polarization of the cell with the formation of a leading edge, which is characterized by lamellipodia that serve as a moving apparatus, and a uropod in the hind part. Interestingly, P-selectin glycoprotein ligand (PSGL)-1 (also known as CD162) is concentrated at the uropod of migrating neutrophils. Circulating P-selectin-expressing activated platelets frequently bind these neutrophils for few seconds and then detach. The most interesting aspect of this phenomenon is that the crawling of neutrophils is dramatically hampered when the mechanisms controlling neutrophil polarization are 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].

> 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

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 physiologic 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 GPVI 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 mainly 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 megakaryo-cytes 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 (sPLA$_2$-IIA) and 12-lypoxygenase (12-LO). sPLA$_2$-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. sPLA$_2$-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 B$_4$ receptors 1 and 2 (LTB$_1$ and LTB$_2$) 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 parodontitis 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 sPLA$_2$-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 lympho-monocytes 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

lymphocytes, against pathogens and autoantigens. Indeed, the binding of platelets to T-cells can be mediated by various receptor-ligand interactions, such as CD40/CD40L and P-selectin/PSGL-1 [26]. Platelets have also been shown to bind to circulating monocytes via the receptor CD147, creating aggregates that are more abundant in RA patients compared to healthy controls. In addition, following their interaction with T cells, platelets are able to induce the secretion of pro-inflammatory cytokines [27].

Some data on the interaction between platelets and lymphocytes are available in the context of infectious diseases, such as HBV hepatitis. In a murine model of HBV liver infection, platelets were shown to significantly contribute to CD8+ T cells homing toward liver cells by adhering to hepatic sinusoid endothelial cells expressing hyaluronic acid residues via CD44. Platelets were then able to bind circulating CD8+ cells, thus facilitating their extravasation towards the hepatocytes [28]. They also facilitate trafficking of lymphocytes toward lymphoid organs via the interaction of platelet-expressed receptor C-type lectin domain family 1 member B (CLEC1B) with podoplanin (also known as gp38), expressed by lymphatic endothelium and T-zone stromal cells [28,29].

Evidence is accumulating that platelets may also have a role in modulating proliferation and differentiation of T helper cells, exerting differential effects on their subtypes Th1, Th2, Treg, and Th17. The studies published on this topic to date are still rare, involve cells and platelets from healthy donors, and use variable experimental methodologies; 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 diseases that mainly target (directly or indirectly) T-cell mediated processes.

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 $\alpha2\beta1$ 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 in 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 tryrosine-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].

Similar to the interplay between neutrophils and platelets that leads to the production of pro-inflammatory prostanoids, FLSs and platelets may also dynamically interact to release lipid mediators. In particular, Boilard and colleagues [35] demonstrated another two-step process involving platelets, which are able to synthesize prostaglandin (PG) H_2 from AA in a COX-1 dependent reaction. However, platelets cannot metabolize PGH_2 any more due to the lack of the enzymatic machinery required to convert it to PGI_2, also known as prostacycline. PGI_2 is well known to be central in the induction of arthritis in the murine K/BxN model and to be significantly increased in the SF of RA patients. Nonetheless, the second catalytic step can be provided by FLSs which indeed bear the enzyme PGI_2 synthase.

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

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.

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References

1. Boilard E, Blanco P, Nigrovic PA. Platelets: active players in the pathogenesis of arthritis and SLE. *Nat Rev Rheumatol* 2012; 8 (9): 534-42.

2. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. *Lancet* (*London, England*) 2016; 388 (10055): 2023-38.

3. Zufferey A, Speck ER, Machlus KR, et al. Mature murine megakaryocytes present antigen-MHC class I molecules to T cells and transfer them to platelets. *Blood Adv* 2017; 1 (20): 1773-85.

4. Linge P, Fortin PR, Lood C, Bengtsson AA, Boilard E. The non-haemostatic role of platelets in systemic lupus erythematosus. *Nat Rev Rheumatol* 2018; 14 (4): 195-213.

5. Zhi H, Dai J, Liu J, et al. Platelet activation and thrombus formation over IgG Immune complexes requires integrin αIIbβ3 and Lyn Kinase. *PLoS One* 2015; 10 (8): e0135738.

6. Koivula M-K, Heliövaara M, Rissanen H, et al. Antibodies binding to citrullinated telopeptides of type I and type II collagens and to mutated citrullinated vimentin synergistically predict the development of seropositive rheumatoid arthritis. *Ann Rheum Dis* 2012; 71 (10): 1666-70.

7. Stack JR, Madigan A, Helbert L, et al. Soluble glycoprotein VI, a specific marker of platelet activation is increased in the plasma of subjects with seropositive rheumatoid arthritis. *PLoS One* 2017; 12 (11): e0188027.

8. Zhang Q, Vignali DAA. Co-stimulatory and co-inhibitory pathways in autoimmunity. *Immunity* 2016; 44 (5): 1034-51.

9. Fu H, Hu D, Zhang L, Tang P. Role of extracellular vesicles in rheumatoid arthritis. *Mol Immunol* 2018; 93: 125-32.

10. Cafaro G, Alunno A, Valentini V, et al. The onset site of rheumatoid arthritis: the joints or the lung? *Reumatismo* 2016; 68 (4).

11. Nurden A. Platelets, inflammation and tissue regeneration. *Thromb Haemost* 2011; 105 (S 06): S13-33.

12. Andia I, Sánchez M, Maffulli N. Joint pathology and platelet-rich plasma therapies. *Expert Opin Biol Ther* 2012; 12 (1): 7-22.

13. Yeo L, Adlard N, Biehl M, et al. Expression of chemokines CXCL4 and CXCL7 by synovial macrophages defines an early stage of rheumatoid arthritis. *Ann Rheum Dis* 2016; 75 (4): 763-71.

14. Sreeramkumar V, Adrover JM, Ballesteros I, et al. Neutrophils scan for activated platelets to initiate inflammation. *Science (80-)* 2014; 346 (6214): 1234-8.

15. Cloutier N, Paré A, Farndale RW, et al. Platelets can enhance vascular permeability. *Blood* 2012; 120 (6): 1334-43.

16. Boilard E, Nigrovic PA, Larabee K, et al. Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science* 2010; 327 (5965): 580-3.

17. Marcoux G, Duchez A-C, Cloutier N, Provost P, Nigrovic PA, Boilard E. Revealing the diversity of extracellular vesicles using high-dimensional flow cytometry analyses. *Sci Rep* 2016; 6 (1): 35928.

18. Molteni M, Gemma S, Rossetti C. The role of Toll-like receptor 4 in infectious and noninfectious inflammation. *Mediators Inflamm* 2016; 2016: 6978936.

19. Fischer A, Abdollahi-Roodsaz S, Böhm C, et al. The involvement of Toll-like receptor 9 in the pathogenesis of erosive autoimmune arthritis. *J Cell Mol Med* 2018; 22 (9): 4399-409.

20. Raijmakers R, van Beers JJBC, El-Azzouny M, et al. Elevated levels of fibrinogen-derived endogenous citrullinated peptides in synovial fluid of rheumatoid arthritis patients. *Arthritis Res Ther* 2012; 14 (3): R114.

21. Kim S-J, Jenne CN. Role of platelets in neutrophil extracellular trap (NET) production and tissue injury. *Semin Immunol* 2016; 28 (6): 546-54.

22. Cafaro G, McInnes IB. Psoriatic arthritis: tissue-directed inflammation? *Clin Rheumatol* 2018; 37 (4): 859-68.

23. Cunin P, Penke LR, Thon JN, et al. Megakaryocytes compensate for Kit insufficiency in murine arthritis. *J Clin Invest* 2017; 127 (5): 1714-24.

24. Cafaro G, Bartoloni E, Alunno A, Gerli R. Platelets: a potential target for rheumatoid arthritis treatment? *Expert Rev Clin Immunol* 2018: 1-3.

25. Duchez A-C, Boudreau LH, Naika GS, et al. Platelet microparticles are internalized in neutrophils via the concerted activity of 12-lipoxygenase and secreted phospholipase A2-IIA. *Proc Natl Acad Sci U S A* 2015; 112 (27): E3564-73.

26. Łukasik ZM, Makowski M, Makowska JS. From blood coagulation to innate and adaptive immunity: the role of platelets in the physiology and pathology of autoimmune disorders. *Rheumatol Int* 2018; 38 (6): 959-74.

27. Rong M, Wang C, Wu Z, et al. Platelets induce a proinflammatory phenotype in monocytes via the CD147 pathway in rheumatoid arthritis. *Arthritis Res Ther* 2014; 16 (6): 478.

28. Iannacone M. Platelet-mediated modulation of adaptive immunity. *Semin Immunol* 2016; 28 (6): 555-60.

29. Del Rey MJ, Faré R, Izquierdo E, et al. Clinicopathological correlations of podoplanin (gp38) expression in rheumatoid synovium and its potential contribution to fibroblast platelet crosstalk. *PLoS One* 2014; 9 (6): e99607.

30. Gerdes N, Zhu L, Ersoy M, et al. Platelets regulate CD4+ T-cell differentiation via multiple chemokines in humans. *Thromb Haemost* 2011; 106 (2): 353-62.

31. Zamora C, Cantó E, Nieto JC, et al. Binding of platelets to lymphocytes: a potential anti-inflammatory therapy in rheumatoid arthritis. *J Immunol* 2017; 198 (8): 3099-108.

32. Zamora C, Cantó E, Nieto JC, et al. Functional consequences of platelet binding to T lymphocytes in inflammation. *J Leukoc Biol* 2013; 94 (3): 521-9.

33. Hsu J, Gu Y, Tan S-L, Narula S, DeMartino JA, Liao C. Bruton's Tyrosine kinase mediates platelet receptor-induced generation of microparticles: a potential mechanism for amplification of inflammatory responses in rheumatoid arthritis synovial joints. *Immunol Lett* 2013; 150 (1-2): 97-104.

34. Lv J, Wu J, He F, Qu Y, Zhang Q, Yu C. Development of Bruton's tyrosine kinase inhibitors for rheumatoid arthritis. *Curr Med Chem* 2018; 25 (42): 5847-59.

35. Boilard E, Larabee K, Shnayder R, et al. Platelets participate in synovitis via Cox-1-dependent synthesis of prostacyclin independently of microparticle generation. *J Immunol* 2011; 186 (7): 4361-6.

36. Itoh Y. Metalloproteinases in rheumatoid arthritis: potential therapeutic targets to improve current therapies. *Prog Mol Biol Transl Sci* 2017; 148: 327-38.

37. Lefevre S, Knedla A, Tennie C, et al. Synovial fibroblasts spread rheumatoid arthritis to unaffected joints. *Nat Med* 2009; 15 (12): 1414-20.

38. Yan S, Yang B, Shang C, et al. Platelet-rich plasma promotes the migration and invasion of synovial fibroblasts in patients with rheumatoid arthritis. *Mol Med Rep* 2016; 14 (3): 2269-75.

39. Alunno A, Falcinelli E, Luccioli F, et al. Platelets contribute to the accumulation of matrix metalloproteinase Type 2 in synovial fluid in osteoarthritis. *Thromb Haemost* 2017; 117 (11): 2116-24.

40. Lee J-C, Min HJ, Park HJ, Lee S, Seong SC, Lee MC. Synovial membrane-derived mesenchymal stem cells supported by platelet-rich plasma can repair osteochondral defects in a rabbit model. *Arthroscopy* 2013; 29 (6): 1034-46.

Capsule

Building a safer cytomegalovirus vector

Vaccine vectors based on cytomegalovirus show strong T cell induction and protection against a multitude of pathogens. However, such vaccines may not be safe for people who are immunodeficient or immunosuppressed. **Marshall** and co-authors genetically modified rhesus cytomegalovirus to allow engagement of host intrinsic immunity. The modified ΔRh110 vector did not spread once administered to nonhuman primates but still induced robust T cell immunity. **Hansen** and colleagues showed in a simian immunodeficiency virus challenge model that the ΔRh110 vector provided durable protection that was comparable to the parental vector. Mutations in the human cytomegalovirus vector could thus yield a potent but restrained cytomegalovirus for human vaccination.

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Eitan Israeli

Capsule

A "sUPR" target for pain management?

The unfolded protein response (UPR) is initiated when unfolded or misfolded proteins accumulate in the endoplasmic reticulum. One highly conserved arm of the UPR, the IRE1α–XBP1 signaling pathway, also plays a role in various other UPR-independent processes, including hypoxia, angiogenesis, and inflammation. **Chopra** et al. reported that this pathway also regulates the production of two molecules, cyclooxygenase 2 and microsomal prostaglandin E synthase 1. These molecules help mediate inflammation-induced pain. When elements of the IRE1α–XBP1 signaling pathway were knocked out, pain behaviors were reduced in two different mouse models of pain. Targeting this pathway may result in improved pain management therapies.

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Eitan Israeli

"It must be remembered that the purpose of education is not to fill the minds of students with facts... it is to teach them to think"

Robert Hutchins (1899–1977), American educator