Platelets - cells with many functions

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Mentor: dr.sc Antonija Jurak Begonja

SVEUČILIŠTE U RIJECI
ODJEL ZA BIOTEHNOLOGIJU
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Karla Soldatić

TROMBOCITI – STANICE S MNOGOBROJNIM FUNKCIJAMA

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ABSTRACT

Platelets are the second most abundant type of blood cells. The range of their functions is wide, but they are best known for their role in haemostasis. Through delicate mechanisms of activation, adhesion and aggregation, platelets successfully repair endothelial injuries, thus stopping bleeding. There are mechanisms in place which regulate activation and inhibition of platelets depending on the stimuli received from their surroundings. When these mechanisms are impaired and out of balance, there is a high chance of haemostatic events evolving into thrombotic ones. Thrombosis can lead to a plethora of disease conditions, some of which are the leading causes of death in the world, and the need for their treatment is, therefore, of great significance. This paper will discuss the various complex roles of platelets which prove them to be a crucial cell in haemostatic and non-haemostatic events. Platelets are found at the interface of inflammatory diseases and tumour formation, and their specific roles in these will be discussed in detail. Furthermore, while they have a protective role in immunity, some viruses are able to spread their infection by using platelets as accomplices. Additionally, this paper will also show the potential platelets hold as targets in treating several diseases and conditions. Finally, all the pathways which allow platelets to become activated and serve in haemostasis, also allow for their contribution in development of thrombosis.

KEYWORDS

Platelets; haemostasis; thrombosis; immunity; inflammation; tumour; antiplatelet therapies

SAŽETAK

Trombociti su druga najbrojnija vrsta krvnih stanica. Spektar njihovih funkcija je širok, ali su najpoznatiji po svojoj ulozi u hemostazi. Putem aktivacije, adhezije i agregacije, trombociti uspješno zaustavljaju krvarenje nastalo prilikom ozljede endotela žila. Postoje mehanizmi koji osiguravaju pravovremenu aktivaciju ili inhibiciju trombocita, ovisno o signalima i promjenama koje detektiraju u svojoj okolini. Ukoliko su ti mehanizmi ugroženi i u disbalansu, tada hemostatski procesi prelaze u trombozu. Tromboza može uzrokovati široki spektar bolesti koje su među vodećim uzrocima smrti u svijetu. Ovaj će rad raspraviti razne kompleksne uloge trombocita koje dokazuju njihovu važnost u hemostatskim i ne-hemostatskim fiziološkim događajima. Trombociti se nalaze na čelu nastanka upalnih bolesti i tumora, te će se njihove specifične uloge u ovim procesima razraditi u ovome radu. Nadalje, iako trombociti imaju zaštitnu ulogu u imunom odgovoru, neki virusi koriste baš trombocite kako bi proširili svoju infekciju. Nadalje, ovaj će rad opisati potencijal koji trombociti imaju kao mete u liječenju raznih bolesti. Svi signalni putevi koji omogućavaju trombocitima izvršenje njihovih funkcija u hemostazi, isto tako omogućuju njihov doprinos u razvitku tromboze.

KLJUČNE RIJEČI

Trombociti; hemostaza; tromboza; imunitet; upala; tumor; antitrombocitna terapija

LIST OF ABBREVIATIONS

CD40L	CD40 ligand
COX	Cyclooxygenase
DC-SIGN	Dendritic cell-specific intracellular adhesion molecule-3 grabbing-nonintegrin
DENV	Dengue virus
DTS	Dense tubular system
EAE	Experimental autoimmune encephalomyelitis
ECM	Extracellular matrix
EGF	Epidermal growth factor
EMT	Epithelial to mesenchymal transition
GPIa-IIa	Glycoprotein Ia-IIa
GPIIb-IIIa	Glycoprotein IIb-IIIa
GPIb-V-IX	Glycoprotein Ib-V-Ix
GPVI	Glycoprotein VI
LPS	Lipopolysaccharide
MHC I	Major histocompatibility complex class 1 molecule
MI	Myocardial infarction
MMP	Metalloproteinases
MS	Multiple sclerosis
NET	Neutrophil extracellular trap
NO	Nitric oxide
PAF	Platelet-activating factor
PAR	Protease-activated receptor
PDGF	Platelet-derived growth factor
PE	Pulmonary embolism
PF4	Platelet factor-4
PGI_2	Prostacyclin
PLC	Phospholipase C
PMP	Platelet microparticle
PSGL-1	P-selectin glycoprotein ligand 1

RA	Rheumatoid arthritis
ROS	Reactive oxygen species
SCCS	Surface-connected canalicular system
TCIPA	Tumour cell-induced platelet aggregation
TF	Tissue factor
TGF-β	Tissue growth factor-β
TLR	Toll-like receptor
TXA_2	Thromboxane A_2
VEGF	Vascular endothelial growth factor
vWF	von Willebrand factor

TABLE OF CONTENTS

1. Introduction

1.1. Morphology of Platelets

Platelets are the second most abundant blood cell type after erythrocytes. They are a vital factor in many haemostatic and physiological processes, and are most known for their role in blood clotting which occurs as a response to blood vessel damage. Structurally, these cells are parts of megakaryocyte cytoplasm, precursor cells derived from the bone marrow. The normal platelet count in an adult's body is between 150 and $400x10^9$/L (1). They are anucleate, but are still metabolically active, and have an important role in interacting with their surrounding environment.

Platelets' plasma membrane is a regular lipid bilayer composed of phospholipids and cholesterol. Phospholipids, more specifically phosphatidylinositol, mediates platelet activation as it is a source of arachidonic acid. Arachidonic acid is an unsaturated fatty acid which is converted into eicosanoids prostaglandin and thromboxane A_2 (TXA_2) when the platelet is undergoing activation. During platelet activation, another type of phospholipid, phosphatidylserine points towards the outer platelet surface, and due to its charged nature binds coagulation factor complex VIII and IX, and coagulation factor complex X and V (2). The phospholipids allow for the semi-permeable nature of the plasma membrane to exist. They support internal platelet activation and external plasma coagulation. Cholesterol is crucial for regulating the cell's stability and fluidity. Within the bilayer, glycoproteins and proteoglycans support the glycosaminoglycans, oligosaccharides and glycolipids found on the surface. These make up the glycocalyx. The glycocalyx allows the platelet to carry its functional environment with it, all while it maintains a negative charge thereby repelling other platelets and endothelial cells (2).

Platelets' plasma membrane protrudes inwards, thereby creating a surface-connected canalicular system (SCCS) unique to these cells (Fig. 1). The main function of the SCCS is to store the same haemostatic proteins

found in the glycocalyx. Parallel to the SCCS, is the dense tubular system (DTS, Fig. 1). This structure originates from the rough endoplasmic reticulum, and has the ability to sequester Ca^{2+} and carries enzymes crucial for platelet activation, including phospholipase A_2, cyclooxygenase (COX) and thromboxane synthetase which mediate TXA_2 production (2).

Platelets' shape, and more importantly their ability to change shape, determines their responsiveness to an injury. The shape of platelets is described by two main cytoskeletal elements: the marginal band of microtubules and the sub-membrane cortex. When in an inactive state, platelets possess a marginal band composed of a single microtubule that is wound in 8-12 coils. This marginal band takes the form of a peripheral ring and it allows platelets to take a discoid shape. While discoid, their diameter is 2-3 microns (1). Found between the microtubules and the plasma membrane are actin microfilaments which are in charge of platelet contractility and anchorage of glycoproteins and proteoglycans.

Platelets contain different types of granules. Around 50-80 α-granules are found in each platelet (Fig. 1). These contain endocytosed and megakaryocyte-produced proteins which fuse into the SCCS upon platelet activation. Their main function is the aiding of platelet adhesion and aggregation, as well as plasma coagulation (2). Alongside α-granules, dense granules can also be found within platelets. They are the main storage of serotonin and ADP (3). Platelets also contain lysosomes whose main function is thought to be the digestion of the vessel wall matrix components during *in vivo* aggregation, as well as the digestion of debris. However, the functions of lysosomes are yet to be fully proven.

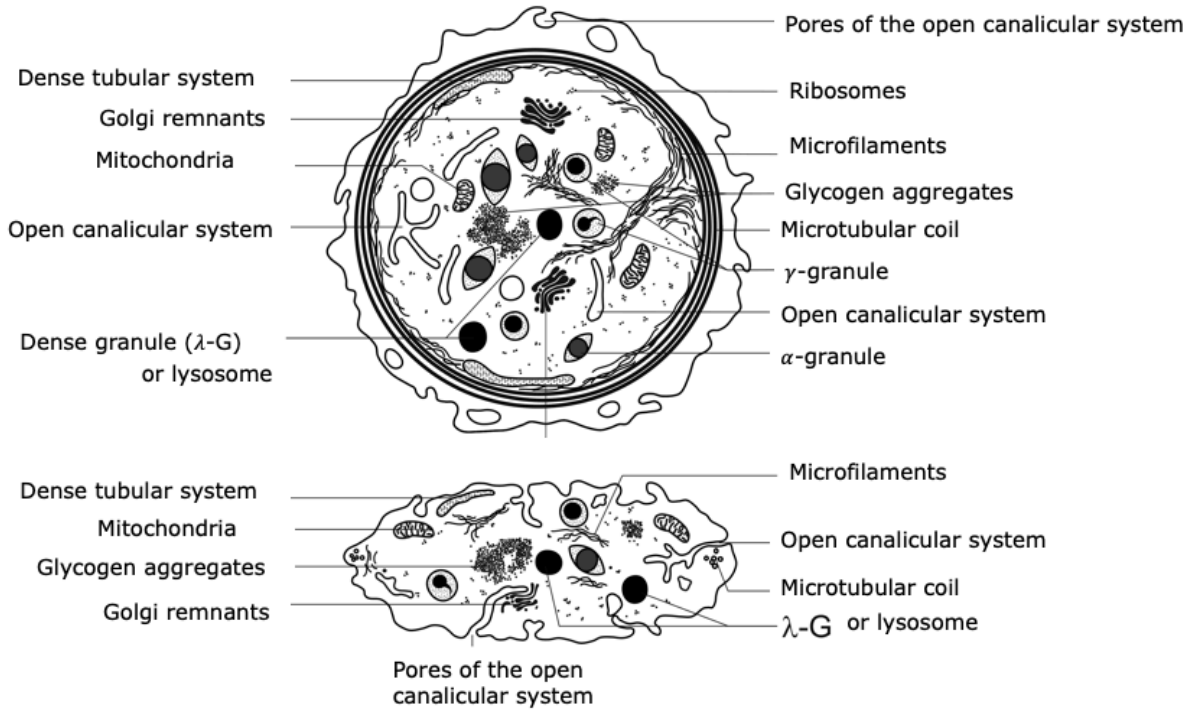

Figure 1: Scheme of platelet cell organelles, equatorial plane and cross-section. Degranulation of α- and dense granules supports adhesion and aggregation. DTS sequesters Ca^{2+} and releases enzymes necessary for activation. OCS or SCCS store proteins needed in haemostasis. Microfilaments and microtubular coil regulate platelet contractility and shape. Lysosomes digest debris. Glycogen aggregates are energy storage. Ribosomes are sites of protein synthesis. Mitochondria are responsible for ATP production and contribute to activation. [Image taken and modified from *(4)*].

1.2. Platelet Receptors Important for Haemostasis

Over 50 receptors can be found within platelets' plasma membrane (2). Most of them are needed for platelet activation, adhesion and aggregation.

Glycoprotein IIb-IIIa (GPIIb-IIIa, intergrin αIIb-βIII) is a fibrinogen receptor responsible for platelet aggregation (Fig. 2). It is the most abundant platelet receptor. It binds fibrinogen and von Willebrand factor (vWF) after it undergoes conformational change as a result of platelet

activation (5). Bound fibrinogen cross connects adjacent platelets at the site of injury and thereby platelets aggregate. GPIIb-IIIa inhibitors like abciximab, tirofiban and eptifibatide are used in treating disorders like the coronary artery disease (CAD) and acute coronary syndromes (ACS), as they are successful in blocking the bond between fibrinogen and the platelet, thus preventing platelet aggregation (6).

Glycoprotein Ib-V-IX (GPIb-V-IX, Fig. 2) is a receptor complex for vWF exposed on the damaged sub-endothelium. It also binds thrombin, P-selectin, factor XI and factor XII. GPIb-V-IX is composed of four subunits; GPIb-α, GPIb-β, GPIX and GPV. They are connected by covalent and non-covalent bonds to make up the quaternary organisation of the transmembrane protein (7). Out of the whole platelet proteome, the Ib beta chain has the highest number of copies: 49000 (8). The GPIb subunit plays a role in the recruitment of leukocytes during inflammation.

P2Y1 and P2Y12 (Fig. 2) are G-coupled purinergic receptors for ADP. They take part in platelet function, haemostasis and thrombosis. Research believes they are crucial in generating a stable haemostatic plug. These receptors facilitate granular release, shape change, ensure the inhibition of adenylyl cyclase and activation of platelets' fibrinogen receptors. Patients with heritable P2Y1 and P2Y12 defects exhibit bleeding abnormalities, and due to their role in platelet aggregation, they are biological targets for treating thromboembolisms (9).

Glycoprotein Ia-IIa (GPIa-IIa, Fig. 2) and glycoprotein VI (GPVI, Fig. 2) are also important for haemostasis. GPVI has 9600 copies (8), mediates cell activation and is a prerequisite for platelets' adhesion, aggregation, degranulation and coagulant activity. It begins to bind to the collagen exposed on the extracellular matrix (ECM) after vessel injury. Once the platelet undergoes the full process of activation, the affinity of GPIa-IIa for collagen rises. The two receptors together cause the platelet to tightly bind to collagen (10).

Thrombin receptors, better known as protease-activated receptors (PAR1-4, Fig. 2), are a family of G protein-coupled receptors (GPCR) (11).

They are activated by proteolysis and, as thrombin is a protease, it has an activating effect on these receptors. All four types of PARs are proven to interact with thrombin, but they vary in their signalling cascades. Some have preassembled complexes with downstream messengers, while others form during activation (11).

TXA_2 binds to thromboxane receptors (TP, Fig. 2), initiating the mobilisation of calcium ions from the DTS. Calcium ions have a stimulatory effect on smooth muscle contraction and actin filaments, thereby contributing to platelet activation (2). Platelet aggregation is further supported through the binding of epinephrine and norepinephrine to α-adrenergic receptors (12).

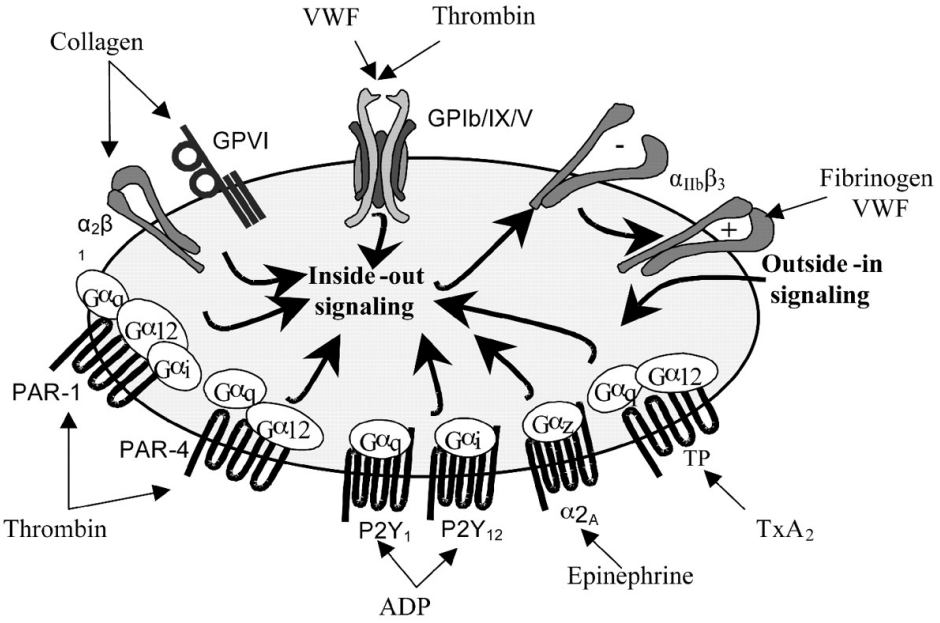

Figure 2: Major platelet receptors. GPIb-IX-V complex binds thrombin and vWF, causing platelet recruitment. GPVI binds collagen and induces adhesion. PAR-1 and PAR-4 bind thrombin. P2Y1 and P2Y12 bind ADP and enhance activation. Epinephrine binds to α-adrenergic receptors causing platelet aggregation. TP receptors bind TXA_2 and cause release of calcium ions. GPIIb-IIIa bind fibrinogen and cause aggregation. [Image taken from *(13)*].

1.3. Other Receptors

As platelets' role is not confined to only haemostatic functions, these cells possess receptors which aid them in performing their thrombotic, immune, inflammatory and tumour functions. Platelet CD40L is essential for communication with other cells, not only in haemostasis, but also in immunity and inflammation. Platelets' P-selectin binds to its ligand on the endothelium and immune cells. Toll-like receptors (TLRs) are believed to be crucial in recognition of pathogens in immune responses. These, some other typical platelet receptors and their copy numbers in human platelets are shown below (Table 1).

Class	Receptor name	Family	Function	Copy number
LRR	GPIb-IX-V complex	Ig and transmembrane	Platelet recruitment	25000
Glycoprotein	GPIIb-IIIa	Transmembrane	Platelet aggregation	60000-100000
	GPVI	Ig	Platelet adhesion	9600
	GPIa-IIa	Transmembrane	Platelet adhesion	4600-10000
Purinergic	P2Y1, P2Y12	GPCR for ADP	Aggregation amplification	n.d.
Thrombin	PAR-1, PAR-4	Transmembrane	Platelet adhesion and spreading	2400
Thromboxane	Thromboxane receptor	Transmembrane and GPCR	Platelet aggregation	n.d.
C-type lectin receptor	CLEC-2	Type II membrane protein C-type lectin receptor	Platelet aggregation	1400
Serotonin	5-HT2	Transmembrane	Thrombus formation	n.d.
Tetraspanins	CD36	Tetraspanin	Stabilization of aggregates	16700
Prostaglandin	PGE_2, PGI_2	GPCR	Inhibition of platelet aggregation	n.d.
P-selectin	P-selectin	Selectin	Clot formation with leukocytes in thrombosis	8900
Antigen-recognizing	FCγRIIA	Ig	Antigen recognition in immunity	1000
	CD40L	TNF superfamily	Platelet granule secretion in immune response	1600
	TLR4	Lipoprotein	Recognition of bacterial PAMPs in immunity	n.d.
	TLR7	Lipoprotein	Recognition of SARS-CoV-2 RNA	n.d.
DC-SIGN	DC-SIGN	Non-integrin	DENV virus binding to platelet	n.d.

Table 1: Platelet receptor classes and their physiological functions. Haemostatic receptors are LRRs, GPs, purinergic, thrombin, thromboxane, CLEC, serotonin, tetraspanin, prostaglandin, P-selectin. Immune receptors are antigen-recognizing and DC-SIGN. [Table made from *(14)*, *(15)* and *(8)*].

1.4. Purpose of Review

The aim of this thesis is to identify and discuss the main functions of platelets, both haemostatic and non-haemostatic. Considering the fact that platelets have an imperative role in a vast range of processes, in most of which their function is not fully understood yet, it is important to discuss these and examine how they interplay. While discussing the roles that platelets are most known for, such as haemostasis, I will also examine the part that platelets play in the immune response, the onset of thrombotic diseases and tumours. A large amount of evidence points to platelets as accomplices in the progression of various conditions, and due to this, more research is being done on their specific contribution. The involvement of platelets is undoubtful in numerous pathophysiological processes and there is a growing need for research on how platelets can be targeted in disease treatment.

2. Homeostasis – Maintenance of Vessel Wall Integrity

Research shows that platelets play a crucial role in maintaining the integrity of blood vessel walls when there are no injuries and no inflammation. According to Gupta *et al* (16), when platelets are depreciated or absent, cells of the vascular endothelium show significant morphological changes. The maintenance of the vessel wall is important because if any blood components manage to pass through the endothelium when this is not wanted, it can lead to the overcompensation of the lymphatic system. This can cause organ dysfunction, and, in some cases sepsis. The role of platelets in homeostasis, specifically, is best understood through the example of vascular leakage caused by thrombocytopenia, a disorder characterised by a low platelet count. The effects of thrombocytopenia take up to four hours to start being showcased. In their experiments, Gupta *et al* (16) utilised fluorescently tagged dextran molecules to monitor for barrier dysfunction of capillaries and postcapillary venules. The rate of extravasation of 40kDA dextran from capillaries and post-capillary venules was shown to be sensitive to circulating platelet count – the extravasation rate was increased in cases of severe thrombocytopenia, where platelet count was <5% of normal, meaning dextran migrated more as the barrier was damaged (16). Dextran numbers began recuperating once the platelet count was reverted to 30% of normal levels, meaning the severity of the barrier damage was lower (16). The experiment showed that there was a reinstatement of thrombocytopenia in mice models which had defects in dense granule release, or were lacking GPVI receptors. This proves that the method of platelets' vessel wall maintenance occurs through the binding of a ligand to GPVI which then initiates a signalling pathway through phospholipase C-b (PLC), finally causing dense granule release (16). However, the type of ligand starting the signalling cascade is still unknown.

3. Haemostatic Functions

3.1.1. Primary Haemostasis

Haemostasis is the physiological response for stopping of bleeding caused by an injury to a blood vessel wall. During an endothelial injury, the subendothelial matrix becomes exposed to the circulating bloodstream containing inactive platelets. This subendothelial matrix is rich in adhesive proteins, one of which is vWF bound to collagen. According to McRae (1), mature vWFs have a heavy molecular weight of up to 20,000,000 daltons, and therefore have to be digested into smaller units by metalloproteinases. Afterwards, the collagen-bound vWF causes a conformational change in the GPIb subunit of the GPIb-V-IX receptor complex, which it can then bind to. As this bond is reversible, there is a strong need for a reaction which will solidify the process. This is ensured by the collagen binding to the GPIa-IIa receptors on platelets. Collagen can also bind to the GPVI receptor. This action further increases platelets' affinity for collagen by stimulating the GPIa-IIa receptor through internal signalling (1).

The requirement of smaller size vWFs ensures that spontaneous platelet activation and aggregation do not occur. Adhesion is the first step towards a platelet being activated. Therefore, vWF acts as a priming factor to other interactions needed for a successful activation pathway to occur (1). The absence of vWF in subendothelial surfaces has shown to cause serious impairments in coagulation, but also haemostasis overall.

Ensuing adhesion, a number of intracellular pathways begin the process of platelet activation. The initial binding of vWF to GPIb and collagen to GPVI triggers a response resulting in shape change, degranulation of α-granules and platelet aggregation (1). The importance of the GPVI receptor, highlighted in most research, lies in the fact that it contains a positively charged arginine in its transmembrane area which non-covalently interacts with a number of kinases. Due to this interaction, the Src family kinases, the Syk kinase and the phosphatidylinositol 3-kinase become activated. Kinase-dependent pathways activate PLC which hydrolyses membrane

phosphatidylinositol 4,5-bisphophate (PIP_2). The cleavage of PIP_2 generates inositol (1,4,5) triphosphate (IP3) and diacylglycerol (DAG). IP3 and DAG are needed for the cytosolic increase of Ca^{2+} and activation of protein kinase C (PKC), respectively (17). PKC phosphorylates cellular proteins and causes granular release and platelet aggregation. Following the IP3-mediated Ca^{2+} release from DTS, four main events occur (1).

The first is the production of arachidonic acid by phospholipase A_2 and PLC. COX-1 and thromboxane synthetase generate TXA_2 (1).

The second event is granular release of both α-granules and dense granules. Dense granules release serotonin and ADP. ADP then binds to P2Y1 and P2Y12. P2Y1 induces shape change, while P2Y12 inhibits adenylyl cyclase, thereby preventing platelets from staying in a state of rest (1). The activation process is consistently maintained through the positive feedback of serotonin, ADP and TXA_2. They are responsible for the recruitment of other platelets to the site of blood loss and activation by binding to their P2Y1 and P2Y12 receptors.

The third event is the activation of fibrinogen receptors GPIIb-IIIa (1). The activation occurs through a conformational change caused by a small GTPase Rap1b. When activated, GPIIb-IIIa can bind fibrinogen from its surrounding environment. Furthermore, this receptor can also bind talin on its tail. Talin intracellularly connects the receptor to actin filaments of the cytoskeleton, causing the amplification of cell adhesion (1).

The last main event caused by an increase in cytosolic Ca^{2+} is the actual shape change of platelets (1). Their new structure resembles pseudopodia. According to Moskalensky *et al* (18), the transition occurs within seconds. The previously mentioned marginal band of microtubules moves out of plane, coils upon activation, and under the influence of cortical tension to form a 3D saddle-like structure. The shape change is explained by an increase in interaction between microtubules of different polarities that are joined together by dynein, a molecular motor protein. As the microtubules are sliding, shortened and elongated regions are formed in the marginal band. The presence of cross-linked protein bridges also plays a role in the

coiling of the marginal band. During experimental observation, the progress of platelet activation can be tracked by examining the stage of curvature that the marginal ring is in (18). These findings are of great importance in understanding platelet migration, adhesion and thrombus formation within a clot.

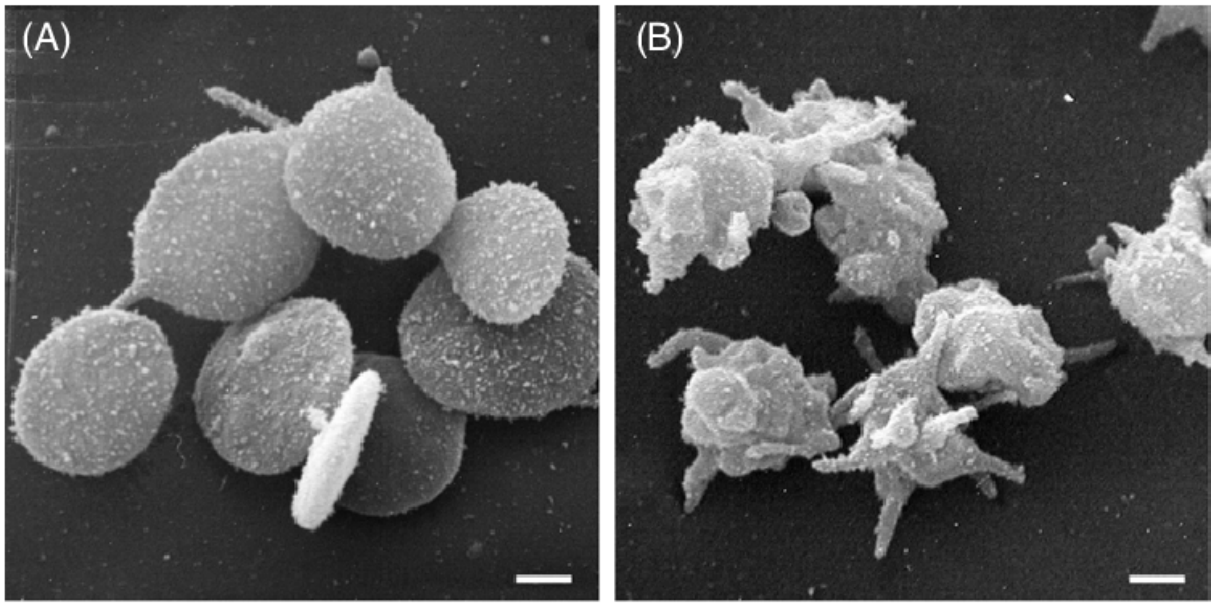

Figure 3: A) Discoid shape of resting platelets vs B) Pseudopodia shape of activated platelets. [Image taken from *(19)*].

The final step of primary haemostasis is platelet aggregation. All of the ligands mentioned in the above section cause the influx of platelets to the site of blood loss, which then undergo irreversible aggregation. Binding of fibrinogen and vWF to GPIIb-IIIa, followed by the cross linkage of GPIIb-IIIa on adjacent platelets form a stable aggregate (1). Following the formation of an aggregate containing platelets, vWF and collagen, primary haemostasis is considered to be finished (Fig. 4).

The main inhibitors of platelet activation are prostacyclin (PGI_2) and nitric oxide (NO) (20). Just like TXA_2 is synthesized by COX-1 and thromboxane synthase from arachidonic acid in platelets, PGI_2 is synthesized by COX-1 and prostacyclin synthase in endothelial cells. NO in endothelial cells is synthesized by nitric synthase. Both of their releases are stimulated by calcium, however NO is released continuously, while

prostacyclin is released in a transient manner (20). In platelets, NO inhibits aggregation, and in smooth muscle it induces vasodilatation. It does so by activating guanylyl cyclase, thereby increasing intracellular cGMP levels. cGMP activates protein kinase G (PKG) resulting in calcium reduction and inhibition of activation (20). Prostacyclin binds to cell surface prostacyclin (IP) receptors. The activation of IP receptors results in G-protein mediated activation of adenylyl cyclase, leading to formation of cAMP. cAMP phosphorylates protein kinase A (PKA), causing intracellular calcium to decrease, and inhibits platelet activation (20).

3.1.2. Secondary Haemostasis

Secondary haemostasis begins during coagulation. As the platelets' changes shape, the contents of its α-granules and lysosomes become exposed and move through the SCCS. Activation and degranulation induce the expression of P-selectin on platelet surface. P-selectin is an adhesive molecule which allows platelets to interact with endothelial cells and leukocytes (1). Other released proteins aid in secondary haemostasis. These are best known as serine proteases and some of them include factor X, XII, XI, and IX. As explained in (21), coagulation can be explained through three main steps.

During initiation, cells found in the non-vascular sub-endothelium express a transmembrane tissue factor (TF). TF binds to factor VII, activates it and mediates the conversion of factor X to Xa, its active form. A portion of factor Xa binds to activated platelets containing TF, while another portion of factor Xa binds to factor Va and converts small amounts of prothrombin into active thrombin (1). Thrombin is mediated by PAR-1 and PAR-4 receptors.

During amplification, the newly produced thrombin binds to the platelets adhered to the endothelium and enhances activation via three bindings sites: one is the GPIb-IX-V receptor complex, one is PAR-1, and one is PAR-4 (22). Binding to GPIb-IX-V significantly increases the affinity of thrombin for PAR-1 and catalysation of platelet activation. Thrombin-mediated

platelet activation induces α-granule release, resulting in high levels of inactive factor V. Thrombin then activates factor V, but also factors VII and XI which lead to further platelet activation (1).

During propagation, highly efficient enzyme complexes, like prothrombinase, are formed. Prothrombinase ensures the conversion of prothrombin into thrombin (1). High concentrations of thrombin are produced, which are now in high enough numbers to cleave fibrinogen into fibrin. Fibrin is essential in making a red, stable blood clot (Fig. 4). During this cleaving reaction, thrombin cuts two fibrinopeptides and exposes a binding site in the central nodule which used to connect the two segments. Through half-staggered reactions, protofibrils align laterally to make fibrin fibres. According to Litvinov *et al* (21), fibrin has viscoelastic properties, meaning it has both reversible elastic characteristics, but can also act like irreversible plastic. Fibrin clots are very durable and easily compressed, therefore can handle a lot of pressure without breaking. Research has shown that the lack of fibrinogen, and therefore fibrin, leads to serious bleeding defects, such as afibrinogenemia and a reduction in life expectancy (21). Fibrinolysis is an essential process for fibrin clearance, as well as the shrinkage of a blood clot and the repair of the vessel's wall as it heals so blood flow can be restored (23).

Coagulation is naturally inhibited by regulatory processes which cause its production to plateau. Some of the most studied under-coagulation disorders, or coagulopathies, are type A and type B haemophilia, which have an x-linked heredity, and the von Willebrand disease, which has autosomal dominant heredity (24). These diseases occur when there is a deficiency in coagulation factors. For haemophilia B, the treatment is factor IX replacement, and VIII replacement for haemophilia A. The treatment for von Willebrand disease is desmopressin, a derivative of an antidiuretic hormone which increases the availability of vWF. Opposingly, hypercoagulability is the increased tendency to form clots in the absence of a vascular injury. These disorders are mainly acquired, however there is growing evidence of the relationship between genetic and environmental

factors causing myocardial infarction and venous thromboembolism (25). These events can occur as a result of congenital antithrombin III deficiency. Antithrombin III binds to heparin on endothelial cells and forms a complex with thrombin, inhibits it from converting fibrinogen to fibrin, and thus prevents coagulation. When antithrombin is deficient, coagulation occurs at a higher rate (25). Hypercoagulability can also occur as a result of a G20210A single nucleotide polymorphism (SNP) in the gene for prothrombin, leading to higher circulation levels of prothrombin and therefore higher levels of thrombin (26). This increases the risk of thrombosis myocardial infarction (25).

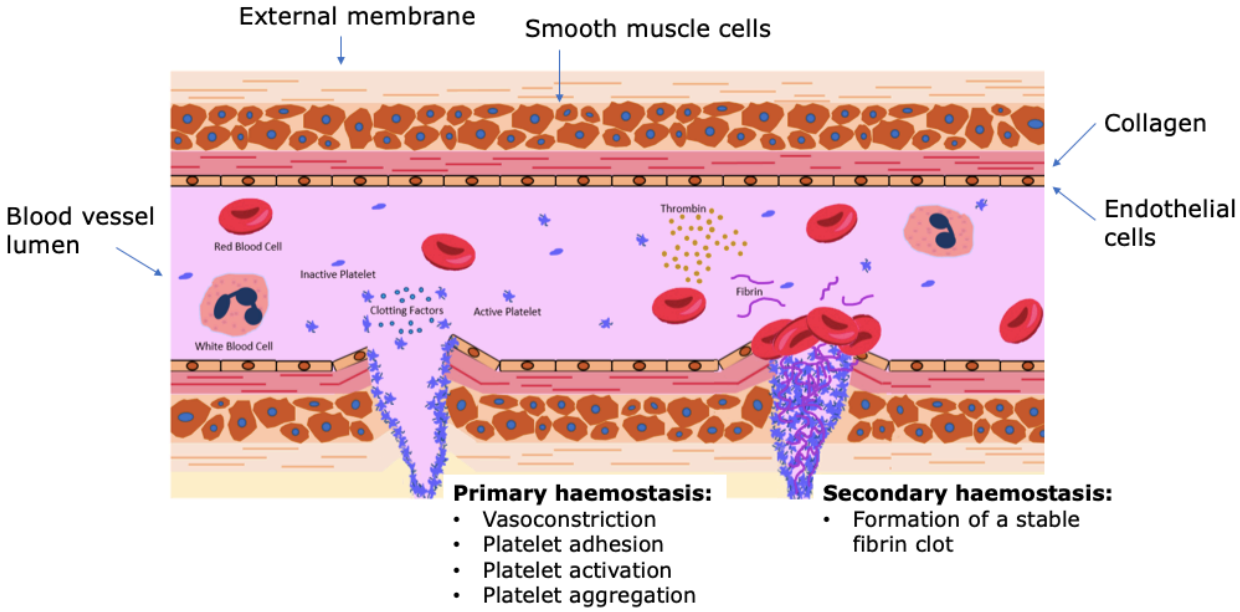

Figure 4: Graphical representation of primary and secondary haemostasis. Vasoconstriction, platelet adhesion, activation and aggregation occur during primary haemostasis. During secondary haemostasis, a stable fibrin clot is formed. [Image taken and modified from *(27)*].

3.2. Thrombosis

Thrombosis is characterized by a clot obstructing blood flow through a vessel that occurs as a result of hypercoagulability induced by changes in

blood flow and blood constituents, and a defective endothelium. Thrombosis includes the inability to control thrombus generation (28). The main participants in thrombosis are aggregates of platelets and endothelial cells. Two main mechanisms facilitate thrombosis: it is collagen or TF-mediated. As already discussed in 3.1.1. and 3.1.2., following endothelial damage, platelets bind collagen and vWF through GPVI and GPIb-V-IX, causing platelet activation and adhesion. Cross-linking of GPIIb-IIIa leads to platelet aggregation, and the secretion of TXA_2, serotonin and ADP while thrombin maintains activation. Thrombosis can also be induced as a result of deep tissue damage which causes the release of TF from smooth muscle, adventitial cells and pericytes. TF then causes the conversion of prothrombin to thrombin and fibrin production (28). The leading cause for arterial thrombosis is atherosclerosis, while venous thrombi are influenced by the Virchow's triad.

Venous thrombosis occurs as a result of three factors: hypercoagulability, changes in blood flow and endothelial injury. These are described as Virchow's triad (28). Venous clots occur in slow shear flow, and are rich in fibrin and erythrocytes (Fig. 5). These may occur even if the vessel's endothelium is healthy. Due to this, venous clots can easily dislocate and travel through the bloodstream, thereby causing pulmonary embolism (PE). The endothelium becomes activated and binds platelets and leukocytes. TF is expressed and the coagulation cascade is activated. Due to low blood flow, hypoxic conditions occur and leukocytes attachment in increased. Erythrocytes change shape which further solidifies the clot. Venous thrombi are treated with coagulation inhibitors (28).

Arterial clots are rich in platelets and form around atherosclerotic plaques in high shear blood flow (Fig. 5) (29). Platelet-leukocyte aggregates (PLA) cause repeated exposure to systemic injurious stimuli. They advance inflammatory processes and contribute to the onset of atherosclerosis (30). They do so by releasing RANTES and platelet factor-4 (PF4) which recruit other innate immunity cells, like monocytes and macrophages to the site of arterial inflammation. Macrophages release pro-inflammatory cytokines and

cause leakage of low-density lipoproteins. Eventually, it evolves into plaques that may obstruct blood flow in arteries and can lead to myocardial infarction (MI) (29). Healthy endothelial cells prevent thrombosis by hydrolyzing ADP to adenosine using enzymes CD39 and CD73. However, in atherosclerotic wounds, endothelial cells lose their regulatory ability of NO and prostacyclin release. Protein disulfide isomerases (PDI) interact with nitric oxide (NO) and reactive oxygen species (ROS), as well as activate TF, making them crucial for thrombi formation. Arterial thrombosis is treated with inhibitors of platelet activation and aggregation (29).

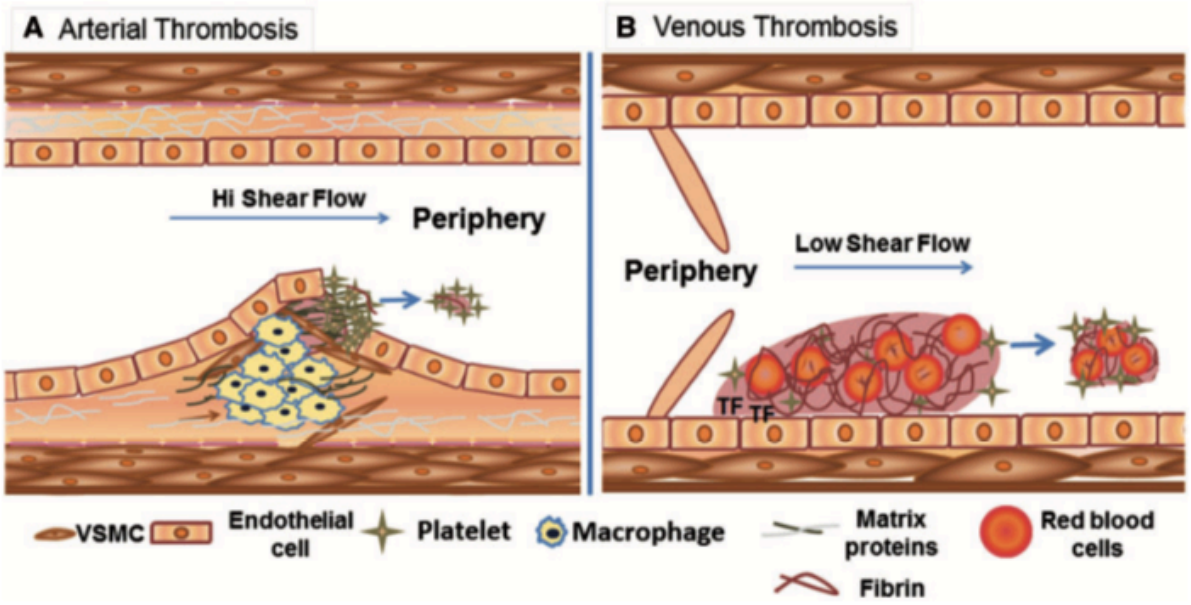

Figure 5: A) Arterial thrombi occur in high shear flow and are rich in platelets. B) Venous thrombi occur in low shear flow, are TF-mediated and rich in fibrin. [Image taken from *(28)*].

The most commonly prescribed drug for secondary prevention of arterial thrombosis is acetylsalicylic acid (Aspirin, Andol). It is a permanent inhibitor of cyclooxygenase thereby preventing the formation of TXA_2. It has been suggested that acetylsalicylic acid is efficient in treating venous thrombi as well. Furthermore, acetylsalicylic acid has a very safe profile, is cost effective and does not need monitoring, making it more suitable than other

anticoagulants (31). Other platelet inhibitors and anticoagulant drugs used to treat thrombosis are shown in Table 2.

Recently, precision medicine has become more important in determining cardiovascular diseases. As platelet mRNA and microRNA are disease-specific markers, platelet proteomics could use them for disease assessment (32).

Antithrombotic agents	**Class**	**Drug name**
Platelet inhibitors	P2Y12 inhibitor	Clopidogrel Prasugrel
	GPIIb-IIIa inhibitor	Abciximab
	Cyclooxygenase inhibitor	Acetylsalicylic acid (Aspirin, Andol)
	PAR-1 antagonist	Vorapaxar
Anticoagulants	Vitamin K antagonist	Coumarin
	Antithrombin III activator	Heparin
	Thrombin inhibitor	Dabigatran

Table 2: Therapies for arterial and venous thrombosis. Platelet inhibitors: Clopidogrel and Prasugel are P2Y12 inhibitors, Abciximab is a GPIIb-IIIa inhibitor, Aspirin is a COX inhibitor, Vorapraxar is a PAR-1 inhibitor. Anticoagulants: Coumarin is a vitamin K antagonist, Heparin is an antithrombin III activator, Dabigatran is a thrombin inhibitor. [Table made from *(28)*].

Haemostasis maintains the integrity of blood flow. If normal haemostasis is overwhelmed by pathological factors, it can quickly become a thrombotic process. The line between the two is very thin, and a range of modulatory molecules and receptors have to be closely monitored and

regulated so that blood clots formed to prevent endothelial bleeding are disassembled timely. Furthermore, thrombosis occurs as a result of improper balance between platelets' stimulatory and inhibitory pathways.

4. Non-haemostatic Functions

4.1. The Role of Platelets in Immunity

4.1.1. Innate Immunity

Platelets have an important role in innate immunity because of their interaction with immune cells. They secrete and are sensitive to a plethora of chemokines and cytokines (Fig. 6) described in detail below. These molecules allow them to induce immune responses involving leukocyte-adhesive molecules, pro-inflammatory cytokines and metalloproteinases. Not only can they recruit immune cells, but they also have a direct function in immunity (33).

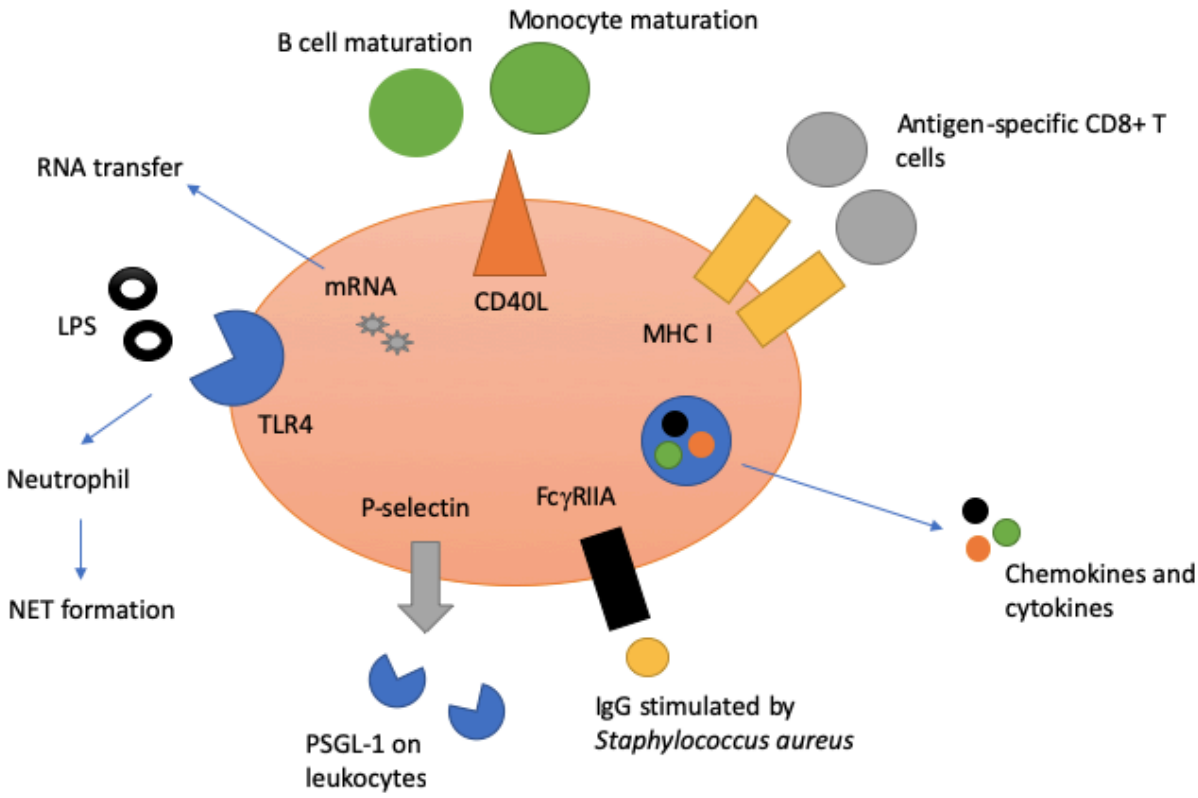

Figure 6: Graphic representation of some of the mechanisms by which platelets take part in both innate and adaptive immunity. Platelets express TLR4 which recognise bacterial LPS and induce NET formation. Release chemokines and cytokines necessary for communication with immune cells. MHC I molecules which are recognised by CD8+ T cells in adaptive immunity. Platelets' P-selectin binds to PSGL-1 on leukocytes and amplifies immune response. IgG binds to platelet FcγRIIA and induces the release of antibacterial proteins. Platelets engage in RNA transfer. CD40L interacts with CD40 on monocytes and B cells and causes maturation.

The role of platelets in innate immunity has been known for decades. Platelets have the ability to recognise bacterial lipopolysaccharide layers (LPS) through their TLR4. This activation by LPS promotes platelet-neutrophil adhesion and formation of neutrophil extracellular traps (NETs) (33). While there are other different types of TLRs found on platelets, their

specific role is still unclear (34). In some cases of LPS recognition, ADP activation of platelets is inhibited. Furthermore, TLR7 activation can lead to thrombocytopenia (32). Due to this, the role of platelet TLRs is still controversial. Upon TLR stimulation, platelets express P-selectin on their surface. P-selectin attracts neutrophils and monocytes and binds to PSGL-1 on their surface.

Platelets' α-granules contain chemokines which attract immune cells to the site of infection, but also thrombocidins, an anti-bacterial protein. Research has shown that these have bactericidal and fungicidal properties, especially against endocarditis caused by *Staphylococcus aureus* (3). Due to this, platelets have an implication in the development of cardiovascular diseases associated with bacterial and viral infections. The methods by which *S. aureus* infection activates platelets are widespread, and it largely depends on the proteins found on the pathogen's cell wall (34). Platelets aggregate around the bacteria which produces α-toxins. In response, platelets release β-defensin which induces NET formation (3).

Regarding viral infections, studies have shown that platelets interact with viruses in various ways. One of them is the promotion of the cytotoxic T lymphocyte against the lymphocytic choriomeningitis virus (LCMV). Platelets recognise LCMV through TLR2 and release interleukin-1β (IL-1β), and CD154 which rapidly help form platelet-neutrophil aggregates (3). These platelets also have an increased interaction with monocytes, B cells and T cells which suggests that there is substantial interplay between the innate and adaptive immunity. However, platelets are not successful in eliminating dengue virus (DENV) infection. DENV is a flavivirus whose positive single-stranded RNA (ssRNA) genome gets directly translated into the cytosol of the host's cells by polyribosome complexes (35). Even though platelets are anucleate, they contain the ribosomes that facilitate this translation. In vitro examination shows that platelets bind directly to DENV's dendritic cell-specific intercellular adhesion molecule-3 grabbing-nonintegrin (DC-SIGN) and, consequently, release its virions, so the infection spreads at an increasing level, as seen in Figure 7. The infection

leads to significant changes in the morphology and haemostatic characteristics of platelets, thus causing thrombocytopenia. This direct translation of DENV RNA into platelets was proven in a study where platelets started expressing viral markers, such as the NS1 protein, even when no viral antibodies were produced, which were, up until that point, believed to be crucial for the interaction between platelets and DENV (35). NS1 is highly immunogenic and because it is secreted into the plasma very early on, it is a marker for early DENV infection.

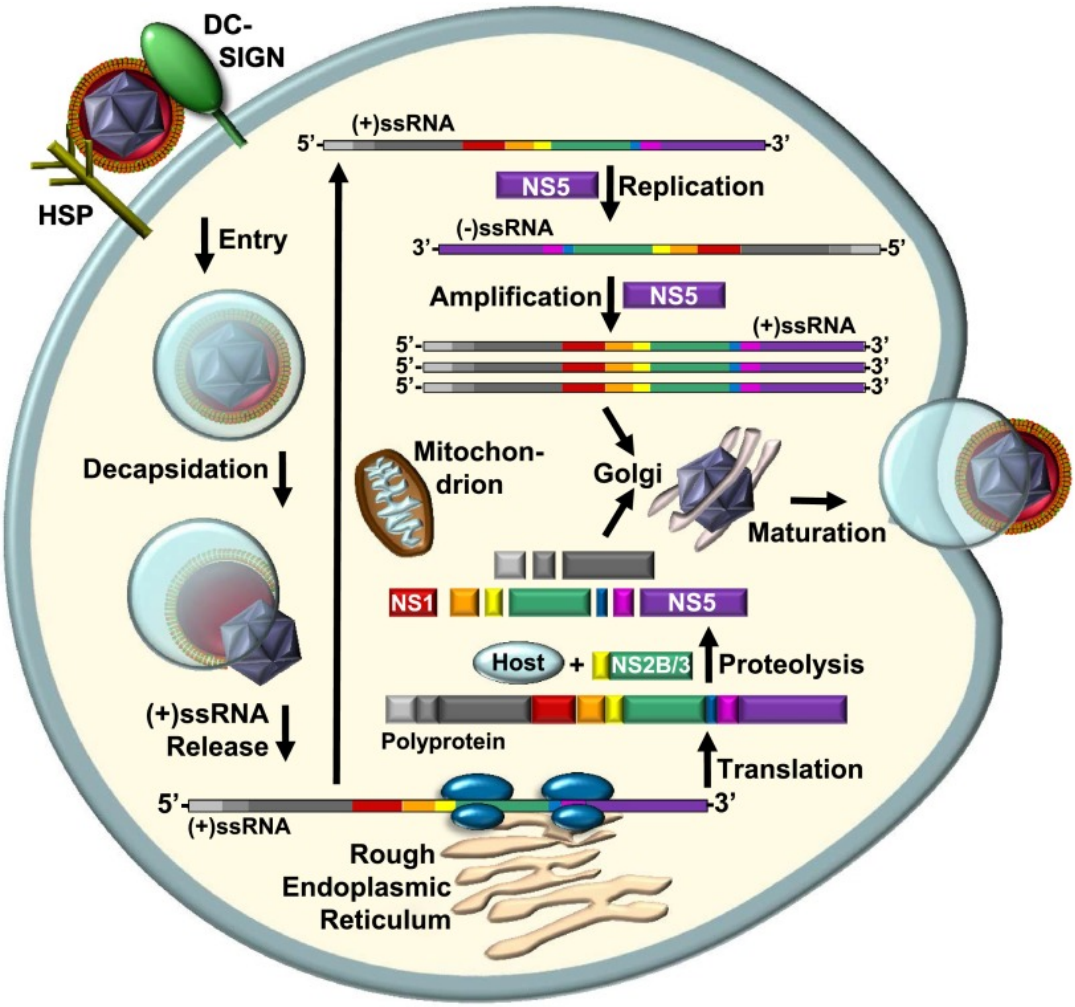

Figure 7: Platelet-mediated replication and translation of DENV RNA. DENV is endocytosed through DC-SIGN and HSP receptors. It is decapsulated and releases its (+)ssRNA that gets translated to produce a polyprotein by ribosomes on the rER. NS5 protein is released from the polyprotein, and mitochondria supply the energy needed for replication and translation of RNA. The virions mature in the Golgi apparatus. NS1 is an immunogenic viral protein. [Image taken from *(35)*].

Regarding Covid-19, this RNA virus is detected by TLR7 on platelets. Platelets' angiotensin-converting enzyme 2 (ACE2) receptor can also detect the viral spike protein (32). This interaction induces the release of PF4 and serotonin, which Covid-19 patients have in high levels (36). PF4 regulates

T cell development, while serotonin causes increased vascular permeability and formation of platelet aggregates. The idea suggesting that platelets contribute to the inflammatory surge and coagulopathy caused by Covid-19 is further supported by high levels of CD40L (36). Coagulopathies examined in Covid-19 patients suggest that antiplatelet therapies, like heparin, could be of importance in reducing the risk of thrombotic disease, and could have anti-inflammatory effects against sepsis (37). Heparin binds to antithrombin III which is a strong anticoagulant. Antithrombin III then inactivates clotting factors, including thrombin and factor Xa. Heparin also blocks P-selectin and reduces the synthesis of interleukins, specifically IL-6 which is found in heightened levels in Covid-19 patients with hypercoagulation (37). Furthermore, heparin is being studied as a direct antiviral agent as it inhibits pathogen binding onto cell surfaces.

4.1.2. Adaptive Immunity

In adaptive immunity, the most important method of platelets communicating with immune cells is through their CD40L, as it has an effect on the induction of maturation of dendritic cells, monocytes, stimulating effect on the production of antibodies by B cells, and increases the activity of T cells (38). This way, platelets play an indirect role in the clearance of an infection.

Platelets deliver antigens to antigen-presenting cells. They are also able to deliver gram-positive bacteria directly to CD8α DCs by binding them to GPIb (39). As this relieves phagocytes of a portion of bacteria they would have to engulf and eliminate, the active delivery mechanism of platelets balances the induction of an antibacterial response and the sterile, homeostatic state of the bloodstream. Due to their ability to deliver pathogens to DCs, platelets play an important role as the communication link between innate and adaptive immunity.

Platelets can also act as antigen-presenting cells. Even though they are anucleate, platelets carry major histocompatibility complex class I (MHC I) molecules on their surface, and also intracellular components needed for an

MHC I-associated pathogen elimination process, such as a proteasome and proteins like calnexin and calreticulin (39).

The exact role of platelets in adaptive immunity remains unclear, however, there is growing evidence of their responsibility in modulating the response (39). Platelets promote the activity of cytotoxic T lymphocytes (CTL) and aide $CD8^+$ T cells in clearing virally infected cells. Not only that, but they also promote the secretion of cytokines that Th1, Th17 and Treg need for the differentiation of immature T cell precursors into CD4 T cells (39). There is a growing interest in their role in promoting B cell proliferation and differentiation, as well as inducing an efficient IgG response. While it has not been fully proven, there is a suggestion that this occurs through platelets linking the T cell to B cell interaction via CD40-CD40L (39).

Platelets also have the ability to communicate with leukocytes and endothelial cells via RNA transfer. This transfer was confirmed in an experiment where platelets carrying mRNA coding for TLR2 were able to transfer the mRNA into leukocytes which were TLR2 deficient (3).

When considering therapeutic treatments for infections, it is important to take the treatments implications on platelets into account. Penicillin, among others, has an anti-platelet function. This is why recent researchers have started to examine platelet antagonists as a potential therapy in treating infections. The IgG receptor FcγRIIA is one example (34). It mediates multiple different responses to bacterial infections, and its antagonists do not jeopardise platelets' haemostatic functions.

4.2. Involvement of Platelets in the Development of Inflammatory Diseases

4.2.1. Multiple Sclerosis (MS)

MS is a neuro-inflammatory disease of the central nervous system (CNS), characterised by the demyelination of neuronal axons. Its onset includes the crossing of the blood-brain barrier by self-reactive leukocytes which then recruit other mediators, including platelets to the site of

inflammation. Platelets release pro-inflammatory cytokines and serotonin, promote activation of astrocytes and microglia, and therefore lead to myelin sheath destruction (30) (Fig. 8). Increased amounts of fibrinogen coagulates and structurally abnormal platelets have been found in MS patients' plaques. The role of platelets in MS was studied in a mouse model of CNS inflammation, where mice were subjected to experimental autoimmune encephalomyelitis (EAE) (40). The study proved increased levels of platelet activation status, as well as high levels of platelet-activating factor (PAF). Mice with genetic ablation of PAF receptors in EAE show a significant reduction of demyelination and inflammation (40). It was suggested that platelet depletion reduces the severity of the disease in mice, and therefore could be beneficial to MS patients, however this is yet to be experimentally proven (30).

Platelet-derived CD40L interacts with endothelial CD40, thereby triggering an inflammatory response. Experimental inhibition of P2Y12 in EAE showed a significant reduction of P-selectin and CD40L, reducing the gravity of the inflammatory response (40). Apart from this, GPIIb-IIIa poses as a promising target in MS treatment.

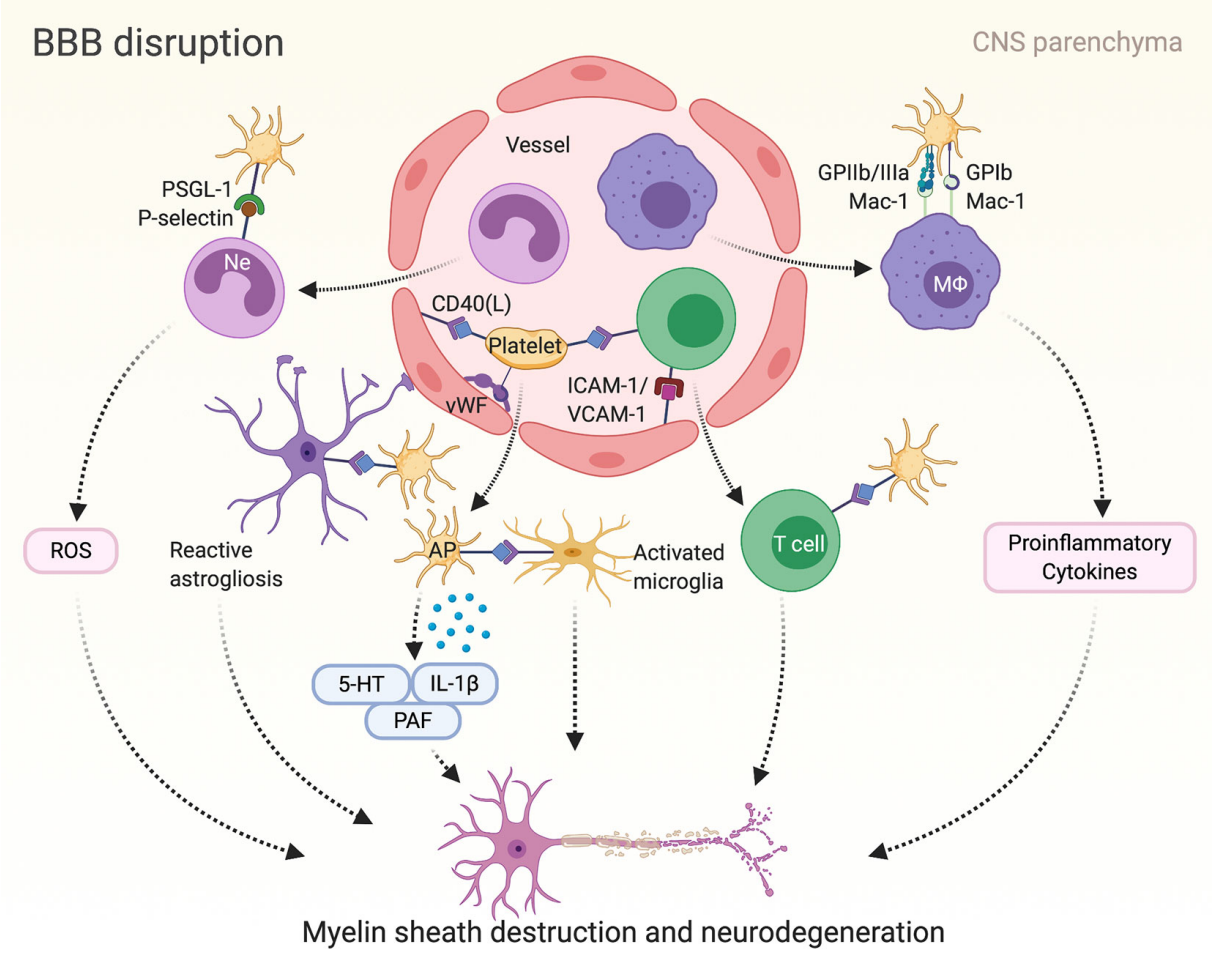

Figure 8: Platelet-mediated inflammation in EAE and MS. Activated platelets (AP) release serotonin (5-HT), interleukin-1β, and PAF, and lead to neuronal damage. Platelets activate microglia and reactive astrocytes causing myelin sheath destruction. By binding P-selectin to neutrophils' PSGL-1, platelets induce ROS secretion and neuronal damage. Binding of platelet GPIIb-IIIa to Mac-1 induces proinflammatory cytokine release from macrophages. Activate T cell destruction of neurons. [Image taken from *(30)*].

4.2.2. Rheumatoid Arthritis (RA)

Rheumatoid arthritis (RA) is an inflammatory disease in which the immune system attacks the host's joints. Aggregates of platelets and leukocytes have been found in blood and synovial fluids of RA patients.

GPIIb-IIIa has a substantial contribution to RA inflammation, and platelet microparticles (PMPs) contribute to chronic synovial inflammation in mice (41). Reduction of platelets in mice leads to decreased bone and cartilage erosion. Unlike MS, the blockage of P2Y12 receptor leads to an aggravated disease course, while the genetic depletion of receptor COX-1 seems to improve symptoms (40).

4.3. Cancer Biology and Platelets

The interaction between tumour cells and platelets is essential for tumour angiogenesis, growth and metastasis. Platelet quantity is used in prognosis and treatment monitoring. In previously undiagnosed patients, a platelet count that is $>3{,}5 \times 10^{11}$/L is a risk marker for cancer. These patients have a 3% chance of cancer within a year of observation (42). Platelet proteome serves as a biomarker for early diagnosis. The expression of platelet-derived proteins greatly changes under tumour signalisation as they are able to sequester tumour-derived proteins. Due to this, patients with early stage lung cancer have significantly increased levels of vascular endothelial growth factor (VEGF) (43). Changes in some other proteins are described in Table 3.

Protein	Early-stage (I-II)	Advanced-stage (III-IV)	Unspecified stage (I-IV)
PDGF	Increased in lung cancer		Increased in colorectal and breast cancer
TGF-β			Increased in breast cancer
VEGF	Increased in lung and breast cancer	Increased in lung, breast, and head of pancreas cancer	Increased in breast, colorectal, hepatocellular, prostate cancer and glioblastoma

Table 3: Increased protein expression in platelets of patients with different cancer types. [Table made from *(43)*].

Platelet mRNA is a newer biomarker method for cancer prognosis and early diagnosis. Platelets do not have a nucleus, so any mRNA they contain is either from megakaryocytes during platelet synthesis, or it is absorbed from interacting with tumours (43).

Blood vessels within a tumour are leaky and discontinuous. They express vWF, collagen and TF. Tumours release ADP and TXA_2, causing platelet adhesion, activation and tumour cell-induced platelet aggregation (TCIPA). Platelets then release a range of bioactive factors, including metalloproteinases (MMP), cytokines and chemokines that support angiogenesis (43). There is an increase in both platelet markers, such as P-selectin, and angiogenesis markers, like VEGF and platelet-derived growth factor (PDGF) which are stored in platelets' α-granules. PMPs are also increased in cancer patients as they deliver angiogenic signals and help form capillary-like networks. P-selectin blockage reduces VEGF expression and the impact of platelets on tumour vascularity (44).

Before detaching from the primary tumour, they induce an epithelial to mesenchymal transition (EMT, Fig. 9) (44). They release podoplanin which

binds to CLEC-2 receptors on platelets. This induces Syk kinase signaling in platelets. The cascade results in the secretion of epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and transforming growth factor β (TGF-β) from platelets' α-granules, thus promoting tumour cell proliferation and invasion. The transition also causes downregulation of E-cadherin, triggering increased cell mobility. At the same time, mesenchymal markers needed for EMT, like Snail and fibronectin, increase. Some suggest CLEC-2 and TGF-β inhibitors could attenuate tumour growth, however not enough evidence is available yet (44). Afterwards, tumour-platelet aggregates promote the nuclear translocation of Yes-Associated Protein 1 (YAP1), and avoid cancer apoptosis known as anoikis. After forming a special microenvironment, tumours can metastasize (42).

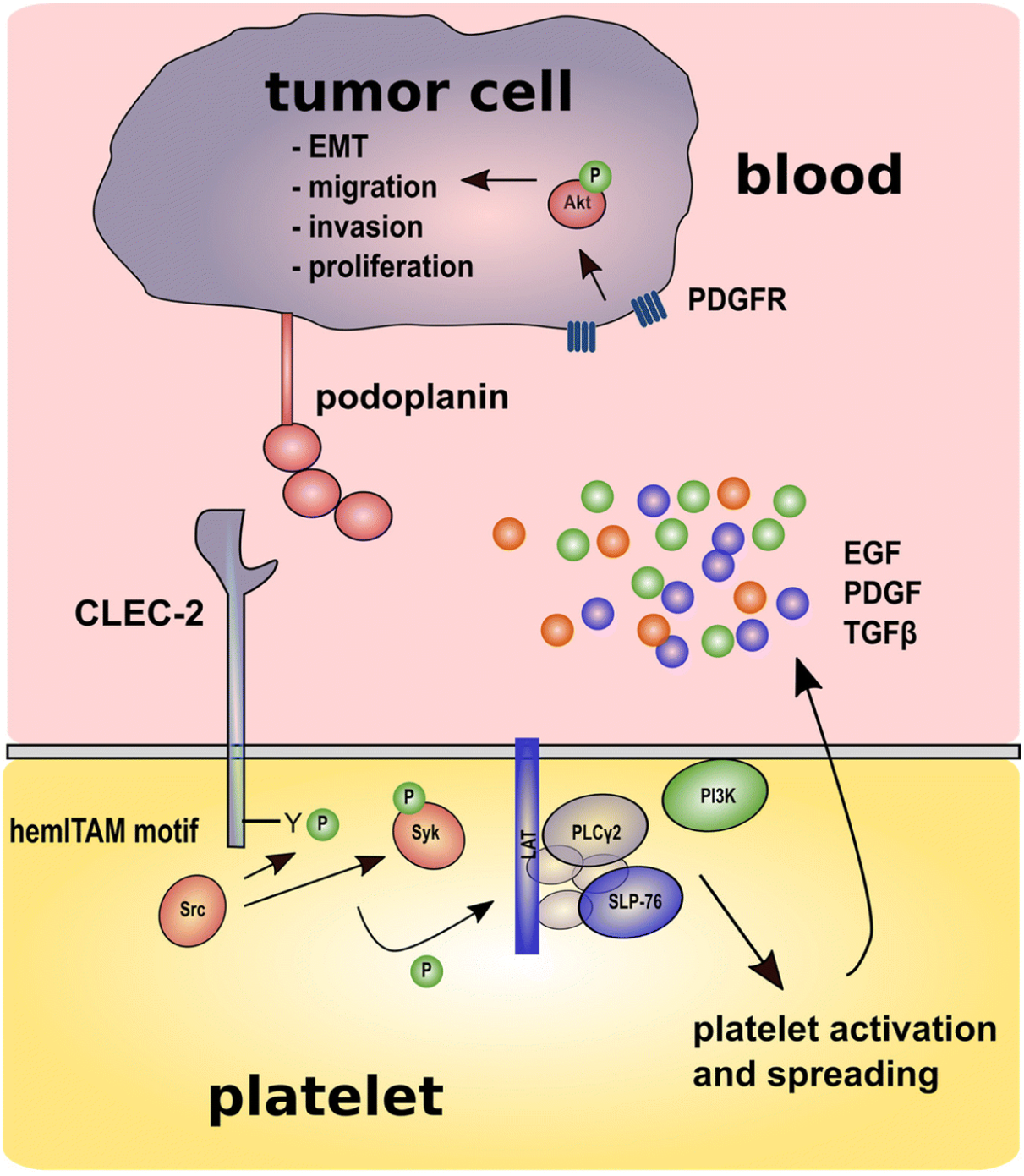

Figure 9: Interaction between tumour cells and platelets to induce an epithelial mesenchymal transition. Tumour cells release podoplanin which binds to CLEC-2 on platelets and induces Syk kinase signaling. Consequently, EGF, PDGF and TGF-β are released from α-granules, promoting tumour proliferation. [Image taken from *(44)*].

Upon leaving the primary tumour and entering the bloodstream, tumour cells are under stress from high shear flow and surveillance by natural killer (NK) cells. Activated platelets encapsulate tumour cells within a thrombus and create a safe and permissive environment. TGF-β secreted from platelets impairs NK cytotoxicity (44). Platelets can also transfer their MHC I onto tumours, making them even harder to detect. P-selectin allows the binding of the platelet-tumour aggregates to endothelium's PSGL-1, and in case of neuroblastoma, induces the activation of pro-survival kinases, thus progressing tumour growth. In case of colorectal cancer, the binding of platelet P-selectin to PSGL-1 on leukocytes induces the human microvascular endothelial cells to express RANTES. RANTES is an inflammatory chemokine recruiting monocytes which then aid in the growth of metastatic foci (44). By doing so, platelets play a crucial role in cancer immunity. Tumour-derived CD97 aids invasion of healthy tissue by promoting platelets' release of pro-inflammatory cytokines and growth factors.

There are many antiplatelet therapies currently being studied as potential cancer treatments. Acetylsalicylic acid, apart from being successful in treating cardiovascular diseases, has anti-cancer characteristics. Inhibition of cyclooxygenases has anti-metastatic and anti-proliferative effects. Low-dose Aspirin combined with clopidogrel reduced the development of tumour cells and deaths caused by hepatocellular carcinoma (45). However, acetylsalicylic acid has other complex inhibitory effects described in Table 4.

Cancer tissue or cell line	**Effect**
Myeloma cell lines	Activation of caspases
SK-OV-3 ovarian and colon	Inhibition of EMT phenotype
Colon and PANC-1	Reduced proliferative potential of cancer cells
Melanoma	Increase in ROS formation and inhibition of tumour growth
Hepatocellular carcinoma	Reduction of immune-mediated pathological effects
Prostate	Induction of apoptosis via NF-κB

Table 4: Aspirin in cancer prevention and treatment. Effect of Aspirin on different cancer tissues or cell lines. [Table made from *(46)*].

Another promising antiplatelet therapy are thrombin receptor antagonists as some of them have been detected in a range of cancers, such as PAR-1, a thrombin receptor. The biggest issue with these drugs is the bleeding risk that they pose, and because of this, they are only a part of clinical trials (45). GPIIb-IIIa antagonists inhibit the final step of platelet aggregation, but can also limit platelet-derived VEGF. Abciximab causes apoptosis of breast cancer cells, while tirofibian reduces the invasiveness of tumour cells. Regarding bone cancer, bone lesions were found in only 4% of GPIIb-IIIa-depleted mice. Opposingly, 74% of mice with a functional GPIIb-IIIa receptor developed osteolytic bone metastasis (46). However, these are also not suitable for long-term use, so there is still a need for the development of further GPIIb-IIIa antagonists (45). One of the novel antiplatelet agents in clinical development is an antiplatelet drug that is a fusion protein of the soluble GPVI with the Fc Ig component. The drug, while

blocking platelet activation by collagen binding, did not affect activation by ADP, therefore no heamostatic functions were endangered. In GPVI-deficient mice with melanoma, there was a 50% reduction in the number of tumour foci (45). All of these drugs, and more, have the potential to be used as therapies of cancer and a number of inflammatory diseases, but, because of their current side effects, they are not suitable for usage in treatment just yet.

5. Conclusion

Platelets are multifaceted cells crucial in many different physiological events. Their predominant function is considered to be haemostasis, where they allow for the repair of a damaged blood vessel wall by cooperating with other cells and modulatory molecules. They are rich reserves of biologically active proteins and, by releasing them, have an effect on a plethora of cells and signaling pathways. There are mechanisms which regulate stimulatory and inhibitory signals, but if an imbalance were to occur, it can lead to defective platelet activation, ultimately causing coagulopathies or thrombosis. Both arterial and venous thrombi lead to cardiac diseases which are currently primary causes of death in the world. Platelets have a vast, but still not fully understood potential in the onset of inflammation, cancers, and pathological diseases, especially the Covid-19 infection. Platelets are a part of an intricate network of mechanisms which allow the spread of viral infections, neuroinflammatory deterioration and cancer growth and metastasis. This review highlights the crosstalk between multiple platelet activation pathways which are beneficial under normal conditions, but are extremely detrimental in cases of inflammatory and tumorigenic progression. Due to this, platelets pose as a target in treatment of various diseases. With emerging new technologies and clinical trials, researchers have made enormous progress in the development of antiplatelet therapies. The main challenge for therapeutic intervention directed at platelets is to find drugs which effectively block specific targets and reduce disease progression, all while leaving at least some of the haemostatic functions intact.

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7. Curriculum vitae

Karla Soldatić

Date of birth: 10/04/1999 | **Nationality:** Croatian | **Gender** Female | (+385) 989254243 | krlsldt@gmail.com |

Nazorova ulica 6, 51211, Matulji, Croatia

WORK EXPERIENCE

01/06/2019 – 01/10/2019 – Opatija, Croatia
TOURIST AGENCY CLERK – LINEAVERDE

I helped organise trips with groups of people, managed the company's website, and made flyers for promotion. I coordinated information with other employees.

04/06/2021 – 18/06/2021 – Rijeka, Croatia
INTERN – NASTAVNI ZAVOD ZA JAVNO ZDRAVSTVO

I worked with various analytical tools including mass spectrometry and gas and liquid chromatography to determine the quality of water, food and air samples. I worked with softwares used to process the data. I also spent time at the Department of Microbiology where I examined human samples.

EDUCATION AND TRAINING

09/2019 – CURRENT – Ulica Radmile Matječić, Rijeka, Croatia
BIOTECHNOLOGY AND DRUG RESEARCH, BACHELOR OF SCIENCE – University of Rijeka, Department of Biotechnology

08/2014 – 05/2018 – Algeria Road, Tripoli Street, Uptown Mirdiff, Dubai, United Arab Emirates
HIGH SCHOOL DIPLOMA – Uptown School Dubai

LANGUAGE SKILLS

Mother tongue(s): CROATIAN | ENGLISH

Other language(s):

	UNDERSTANDING		SPEAKING		WRITING
	Listening	Reading	Spoken production	Spoken interaction	
FRENCH	A1	A2	A1	A1	A2

Levels: A1 and A2: Basic user; B1 and B2: Independent user; C1 and C2: Proficient user

DIGITAL SKILLS

Microsoft Word | Microsoft Excel | Microsoft Powerpoint | Social Media