
Virus–Platelet Associations

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Abstract

Virus–platelet interplay is complex. Diverse virus types have been shown to associate with numerous distinct platelet receptors. This association can benefit the virus or the host, and thus the platelet is somewhat of a renegade. Evidence is accumulating to suggest that viruses are capable of entering platelets. For at least one type of RNA virus (dengue virus), the platelet has the necessary post-translational and packaging machinery required for production of replicative viral progeny. As a facilitator of immunity, the platelet also participates in eradicating the virus by direct and indirect mechanisms involving presentation of the pathogen to the innate and adaptive immune systems, thus enhancing inflammation by release of cytokines and other agonists. Virus-induced thrombocytopenia is caused by tangential imbalance of thrombopoiesis, autoimmunity, and loss of platelet function and integrity.

Introduction

The historical view that platelets solely participate as primary facilitators in hemostasis and thrombosis is now outdated. Reminiscent of roles played by their evolutionary counterparts in primitive organisms (Delvaeye and Conway 2009), it is now well-established that platelets directly modulate cells of the immune system through receptor-mediated cell contact with leukocytes and pathogens. In combination with these interactions, external stimuli induce secretion of cytokines and other cell modulators from platelet α -granules and dense granules to produce additional indirect effects on the cellular immune response. Moreover, in contrast to the

bygone paradigms, the platelet proteome is not static. Platelets inherit from antecedent megakaryocytes a repertoire of mRNAs and the necessary post-transcriptional machinery to alter their protein composition (Schubert et al. 2014). Platelets survey their local environment, reacting dynamically to change, and are thus pivotally positioned during the defense against pathogens (Semple et al. 2011). Here, we focus on their complex interactions with viruses.

Many excellent review articles are available that recognize the importance of platelets in virology. In some cases, these highlight the effects of specific virus types (Chabert et al. 2015; Hottz et al. 2011), whereas others are more general (Assinger et al. 2014; Zapata et al. 2014). The ultimate message is that viruses trigger a complexity of biochemical and cellular events, often resulting in diminished platelet count, altered vascular permeability, and consequent bleeding diathesis. In this review, we revisit these ideas and add a temporal aspect to the emerging model. We speculate that platelets are more than immune modulators and “innocent” bystanders in the host response to viral infection and that, in the case of viruses with an RNA genome, platelets initially participate as a viral ally.

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Virus Binding to Platelets

Platelets are not considered to be a primary host cellular target in virology. Therefore, to identify the receptors that bind viruses, other cell types are usually investigated. The virus–cell interaction is the key initiating step in the virus lifecycle and a logical target for development of antiviral agents. Based on the knowledge of receptors presented on typically studied cells, predictions can be made about whether platelets express the necessary receptors to facilitate specific virus interactions. To help explain platelet-related pathology as a result of infection of certain viruses, direct binding has been reported. Here we loosely divide these interactions on the basis of two principal hemostatic diseases in which direct virus–platelet interactions could logically be involved: thrombocytopenia and hemorrhagic fever (HF).

Thrombocytopenic Viruses When a foreign particle, such as a virus, binds directly to the platelet surface, it is reasonable to speculate that consequent immune recognition leads to a reduction in platelet count. Thus, virus-induced thrombocytopenia has been the rationale for investigating interactions between viruses and platelets. Table 1 lists viruses that correlate with thrombocytopenia without HF. Representing at least six virus families, these viruses range broadly in structure and genome organization and, with the exception of adenoviruses, are surrounded by a lipid bilayer envelope that contains both host cell- and virus-derived elements. Therefore, an adaptive immune response as a result of viral infection of platelets may involve both virus antigens and co-epitopes originating from virus and host factors, resulting in a reduced platelet count. Receptors on the platelet that associate with viruses have been identified and include integrins $\alpha 5\beta 1$, $\alpha_{IIb}\beta_3$, and $\alpha 5\beta 3$; the lectin, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN); Toll-like receptors (TLRs) 2 and 4; coxsackie-adenovirus receptor (CAR); complement receptor 2 (CR2); C-X-C chemokine receptor type 4 (CXCR4); C-type lectin domain family 2 (CLEC-2); chemokine C-C motif ligand (CCL); and glycoprotein (GP) VI. Co-receptor systems involving more than one virus–platelet interaction may also exist, as in human immunodeficiency virus (HIV). In some cases, the platelet receptor is unknown (e.g., SARS-CoV).

A variety of methods have been used to establish specific receptors. Early inhibition studies involving adenovirus used purified matrix proteins, adenovirus penton base proteins, and synthetic peptides. Results suggested the importance of the $\alpha 5$ and $\beta 1/\beta 3$ integrins for infection of several cell lines (Stevenson et al. 1997; Wickham et al. 1993). Immuno- and electron microscopy studies have shown that platelet $\alpha_{IIb}\beta_3$ is also important for the binding of adenovirus (Gupalo et al. 2013). Further cellular studies implicated CAR (Bergelson

et al. 1997) in the virus–platelet association and identified this receptor on platelets by flow cytometry and RNA isolation (Othman et al. 2007). Additional studies using immuno-inhibition and arginyl–glycyl–aspartyl (RGD) motif peptide mimics confirmed the importance of $\alpha 5$ integrins in adenovirus binding to platelets; however, they failed to detect CAR expression (Shimony et al. 2009).

Hepatitis C virus (HCV) is regularly associated with thrombocytopenia (Weksler 2007). Platelet glycoprotein GPVI has been implicated in HCV–platelet interaction through peptide and immuno-inhibition studies (Pugliese et al. 2004), purified protein assays, and virus binding assays (Zahn et al. 2006) and was shown to be important for infection and dissemination (Ariede et al. 2015; Zahn et al. 2006). DC-SIGN has also been demonstrated to be involved in HCV binding.

As in infection of other cell types, HIV has been shown to associate with platelets through a variety of cell-surface receptors (Youssefian et al. 2002). Platelet DC-SIGN, as identified by flow cytometry, western blotting, and PCR, can recognize and bind pathogen-associated molecular patterns (PAMPs) on HIV because immuno-inhibition results in decreased binding (Boukour et al. 2006; Chaipan et al. 2006). Using similar immuno-inhibition and flow cytometric approaches, CLEC-2 was identified as a platelet receptor for HIV (Chaipan et al. 2006). Additional putative receptors for HIV on platelets also include CXCR4, CCL3, and CCL5 (Flaujac et al. 2010).

Platelet integrins appear to serve as the main binding partner because they contain the common RGD motif. Therefore, the presence of integrin-binding sequences in several virus families suggests that integrins are important for platelet association and signaling, with effects leading to thrombocytopenia. Viral envelope glycoproteins can serve as sources of PAMPs and facilitate virus–platelet interaction via TLRs. This is suggested as a mechanism for cytomegalovirus (CMV)-induced thrombocytopenia. Evidence implicating direct binding and consequent cell stimulation as a result of CMV-encoded glycoprotein B and glycoprotein H interactions with TLR2 (Boehme et al. 2006) on platelets or neutrophils has been obtained from immunoprecipitation and immuno-inhibition studies of co-transfected human embryonic kidney and normal fibroblast cells, respectively (Assinger et al. 2014).

Platelets also have on their surface the complement receptor type 2 (CR2), which functions as a receptor for Epstein–Barr virus (EBV), as shown by cell and immuno-inhibition techniques (Ahmad and Menezes 1997; Hutt-Fletcher 2007). Whether this also protects the virus from complement-mediated innate immune clearance is not known.

Platelets have one class of receptor for the Fc domain of antibodies, Fc γ RIIA. Once virus-directed antibodies are generated by the adaptive immune response, “bridged” interactions can be facilitated by platelet Fc γ RIIA, as demonstrated for influenza A virus (IAV) (Boilard et al. 2014).

Table 1 Viruses correlating with thrombocytopenia

Virus	TCP	HF	Family	Genome	Receptor on Platelets	Virus Receptor	Other Cell Receptor	Platelet Entry
Adenovirus (Othman et al. 2007, Shimony et al. 2009, Zhang and Bergelson 2005)	Yes	No	Adenoviridae	DNA, non-enveloped	CAR $\alpha_{11b}\beta_3$ $\alpha_5\beta_3$ $\alpha_5\beta_1$ (?) sialic acid (?) TLR4 (?)	Fiber knob RGD of penton base	$\alpha_5\beta_1$, $\alpha_5\beta_3$, $\alpha_5\beta_5$, TLR3, TLR4, TLR9, sialic acid	Yes
CMV (Agbanyo and Wasi 1994, Assinger et al. 2014, Boehme et al. 2006)	Yes	No	Herpesviridae	DNA, enveloped	TLR2 $\alpha_5\beta_1$	gB, gH	TLR DC-SIGN	ND
EBV (Ahmad & Menezes 1997, Hutt-Fletcher 2007)	Yes	No	Herpesviridae	DNA, enveloped	CR2	gp350/220	CR2	ND
VZV (Rand & Wright 1998, Sloutskin et al. 2014, Zhu et al. 1995)	Yes	No	Herpesviridae	DNA, enveloped	HSP (?)	gE	HSP	ND
HSV (Caviness et al. 2008, Spear 2004)	Yes	No	Herpesviridae	DNA, enveloped	HSP (?)	gC, gD	HSP	ND
Influenza (Boillard et al. 2014, Danon et al. 1959, Le et al. 2015)	Yes	No	Orthomyxoviridae	RNA, enveloped	$\alpha_{11b}\beta_3$, FcγRIIa, sialic acid (?)	HA	Sialic acid FcγRIIa	Yes
HIV (Chaipan et al. 2006, Youssefian et al. 2002, Zapata et al. 2014)	Yes	No	Lentivirus	RNA, enveloped	CCL1,3,4 CXCR4 DC-SIGN CLEC-2	ENV protein	DC-SIGN	Yes
HCV (Ariede et al. 2015, de Almeida et al. 2007, Zahn et al. 2006, Zahn and Allain 2005)	Yes	No	Flaviviridae	RNA, enveloped	DC-SIGN GPVI	E2	DC-SIGN Heparin CD81	ND
SARS-CoV (Imai et al. 2005, Li et al. 2003)	Yes	No	Coronaviridae	RNA, enveloped	CD13	gS	ACE2	ND

Viruses that induce only thrombocytopenia (TCP) and not hemorrhagic fever (HF) are listed. Those viruses that have an RNA genome (*red*) and are permissible to entry may be replicated by platelets. Viruses encoded by a DNA genome (*green*) cannot be replicated by platelets $\alpha N\beta N$ member of the integrin family, *ACE2* angiotensin converting enzyme 2 receptor, *CAR* coxsackie-adenovirus receptor, *CCL* chemokine (C-C motif) ligand, *CD* cluster of differentiation, *CLEC-2* C-type lectin domain family 2, *CMV* cytomegalovirus, *CR* complement receptor, *CXCR4* C-X-C chemokine receptor type 4, *EBV* Epstein-Barr virus, *E* or *ENV* envelope, *DC-SIGN* dendritic cell-specific intercellular adhesion molecule-3-grapping non-integrin, *FcγRII* Fc gamma receptor II, *gp* or *g* glycoprotein, *GPVI* platelet glycoprotein VI, *HA* hemagglutinin, *HCV* hepatitis virus C, *HIV* human immune deficient virus, *HSP* heparan sulfate proteoglycan, *HSV* herpes simplex virus, *ND* data not available in the literature, *RGD* arginine–glycine–aspartic acid peptide sequence, *SARS* severe acute respiratory syndrome, *TLR* Toll-like receptor, *VZV* varicella zoster virus, ? proposed platelet receptor based on indirect cell studies

Making vaccine development difficult, some secondary viral infection mechanisms exploit antibody-dependent enhancement; examples are dengue virus (DENV) and HIV (Guzman et al. 2013). Although antibody-bridged binding to platelets has not been specifically documented for many viruses, it is reasonable to speculate that such viral immune complexes commonly form

interactions with platelets. Similar to engagement of other receptors on the platelet surface, these multivalent adducts could crosslink the FcγRIIA, causing platelet activation.

Hemorrhagic Fever Viruses Whereas the bleeding phenotype inherent to thrombocytopenia is considered to be

predominantly caused by loss of platelets, the effects on hemostasis leading to virus-induced HF are typically far more complicated (Zapata et al. 2014). HF is characterized by the loss of blood homeostasis, leading to increased vascular permeability and bleeding, which can progress to shock. The causative agents of viral HF are enveloped RNA viruses from four families: Flaviviridae, Bunyaviridae, Arenaviridae, and Filoviridae (Table 2). These families contain well-known species such as Ebola and DENV that are featured as “headline news” because of the devastating and graphic illnesses these epidemic pathogens can cause. Some of the viruses are especially difficult to experimentally manipulate because of their biohazard classification level and, consequently, relatively little is known about their biochemistry. Nevertheless, receptors for viruses known to cause viral HF have been identified. Interestingly, these are similar to those receptors characterized for viruses as predominantly thrombocytopenic: $\beta 1/\beta 3$ integrins, lectins, and TLRs. Therefore, it is not surprising that HF viruses can also result in thrombocytopenia.

Using specific antibody inhibition and virological plaque-forming assays, DC-SIGN and heparan sulfate proteoglycan (HSP) were demonstrated to be important in DENV binding to platelets (Hottz et al. 2013b; Simon et al. 2015). Interestingly, additional binding sites were expressed by pretreating platelets with the agonist thrombin (Simon et al. 2015). Whether these are the same type of receptor is unknown. Employing a transduced cell model of infection, DC-SIGN has also been shown to be involved as a receptor in Ebola virus infection (Alvarez et al. 2002). Mutagenesis, binding, and RNA interference experiments have implicated Ax1 (Shimajima et al. 2007) and Tyro3 (Hunt et al. 2011) in Ebola’s complex cell engagement mechanism. These observations suggest that multiple receptors are occupied in the Ebola–platelet interaction. Interestingly, similar platelet surface molecules may bind Lassa virus, as indicated by the identification of DC-SIGN, Ax1, and Tyro3 as receptors using cell binding and infection assays (Shimajima et al. 2012). In addition, the results of immunoblot and competition assays involving purified virus suggested that α -dystroglycan is a Lassa virus receptor (Cao et al. 1998). Neutralizing antibody experiments have implicated $\beta 3$ integrins in the interaction between cells infected with hantavirus and platelets (Gavrilovskaya et al. 1999; Gavrilovskaya et al. 2010). Although cell and platelet receptors remain elusive for some HF viruses, it is possible that a particular receptor may function across an individual virus family.

Virus–Platelet Interactions with Moderate Pathology Although virus–platelet interactions leading to life-threatening effects are well-known, there are also a number of viruses that bind to platelets without serious hematological pathology. Predominately from the Picornaviridae family,

these non-enveloped RNA viruses cause enteric, throat, and nasal infections. Although not directly confirmed as analogous functional receptors for picornavirus on platelets, $\beta 1$ and $\beta 3$ integrins are known to facilitate interactions with receptors on other cell types. Studies following surface expression of mutated proteins in CHO cells and antibody inhibition studies have identified integrin $\alpha 2\beta 1$ as a binding partner for both rotavirus and echovirus (Coulson et al. 1997; Fleming et al. 2011). Utilizing an antigenic inhibition model and CHO transfection, members of the $\beta 1$ and $\beta 3$ integrin families were shown to mediate parechovirus infection (Triantafyllou et al. 2000). This suggests the potential for binding recognition with the integrin known to be expressed on the platelet surface. It is somewhat puzzling that thrombocytopenia is not induced because engagement of these receptors by other viruses is consistent with this outcome. Consequently, integrins may not be the only common denominator resulting in this virus-mediated hemostatic disorder.

Virus-Induced Platelet Activation Platelets circulate in a resting state and are stimulated by ligand–receptor engagement. It is clear that protein and carbohydrate receptors on the surface of platelets bind viruses from many distinct families (Tables 1, 2, and 3). Indeed, it is not surprising that the interactions known to exist between viruses and platelet receptors can facilitate platelet activation. Interactions with DENV grown in culture have been shown to induce platelet shape changes, as monitored by atomic force and electron microscopy (Ghosh et al. 2008). DENV has also been demonstrated to cause the exposure of P-selectin and procoagulant phospholipid on the platelet surface (Hottz et al. 2013b; Simon et al. 2015), and to initiate apoptosis-like markers, including caspase activation and mitochondrial permeability changes (Hottz et al. 2013b). Similarly, HIV can induce microparticle release and other hallmarks of platelet activation (Wang et al. 2011) that are related to plasma virus levels (Mayne et al. 2012). Differential response to platelet agonists in HIV-infected individuals points to functional changes in platelets, with implications for thrombosis (Satchell et al. 2010). Interestingly, additional studies further implicated HIV treatment strategies in the development of hyper-responsive platelets (Falcinelli et al. 2013; Gresele et al. 2012). Although the direct effect of virus binding on platelet activation has not been studied extensively, outside-in signal-induced platelet changes are probably a general characteristic, regardless of the type of virus.

Virus Replication by Platelets

Entry Cell binding is the first step used by all viruses to exploit the host’s cellular replication apparatus. Without

Table 2 Hemorrhagic fever viruses with putative platelets interactions

Virus	TCP	HF	Family	Genome	Receptor on Platelet	Virus Receptor	Other Cell Receptor	Platelet Entry
Dengue virus (Noisakran et al. 2009, Simon et al. 2015, Tassaneeritthep et al. 2003)	Yes	Yes	Flaviviridae	RNA, enveloped	$\alpha_5\beta_3$ DC-SIGN HSP FcγRIIa,	GPE	DC-SIGN, LSIGN	Yes
Yellow Fever	ND	Yes	Flaviviridae	RNA, enveloped				ND
Kyasanur forest disease virus)	ND	Yes	Flaviviridae	RNA, enveloped				ND
Alkhumra virus	ND	Yes	Flaviviridae	RNA, enveloped				ND
Hantavirus (Gavrilovskaya et al. 1999, Gavrilovskaya et al. 2010)	Yes	Yes	Bunyaviridae	RNA, enveloped	$\alpha_{IIb}\beta_3$, $\alpha_5\beta_1$ (?)	GN, GC	$\alpha_{IIb}\beta_3$, $\alpha_5\beta_3$, $\alpha_5\beta_1$	ND
Rift valley fever (de Boer et al. 2012)	ND	Yes	Bunyaviridae	RNA, enveloped	HSP (?)		HSP	ND
Severe fever with thrombocytopenia syndrome (Jin et al. 2012)	Yes	Yes	Bunyaviridae	RNA, enveloped				ND
Crimean–Congo hemorrhagic fever	Yes	Yes	Bunyavirus	RNA, enveloped				ND
Chikungunia virus (Long et al. 2013)	ND	Yes	Togaviridae	RNA, enveloped		GP	DCIR	ND
Lassa (Kunz et al. 2005, Shimojima et al. 2012, Zapata et al. 2014)	ND	Yes	Arenaviridae	RNA, enveloped	DC-SIGN	GPC	α - DG Tyro3, DC-SIGN, Axl,	ND
Lujo	ND	Yes	Arenaviridae	RNA, enveloped				ND
Machupo	ND	Yes	Arenaviridae	RNA, enveloped				ND
Guanarito	ND	Yes	Arenaviridae	RNA, enveloped				ND
Junin	Yes	Yes	Arenaviridae	RNA, enveloped				ND
Ebola (Alvarez et al. 2002, Shimojima et al. 2007, Zapata et al. 2014)	Yes	Yes	Filoviridae	RNA, non-enveloped	DC-SIGN (?) $\alpha_5\beta_1$ (?)	GP	Tyro3, DC-SIGN, Axl, $\alpha_5\beta_1$, NPC1	ND
Marburg	ND	Yes	Filoviridae	RNA, non-enveloped				ND

Viruses that cause hemorrhagic fever (HF) are listed. All known HF viruses are RNA viruses and therefore if entry into platelets is permissible, they can be replicated. Dengue virus is the only one for which evidence of platelet-mediated replication has been investigated. HF viruses that interact with platelets span many virus families, as highlighted by the different colors. Some of these viruses are also known to induce thrombocytopenia (TCP) and, although data is not available in the literature (ND) for each, it cannot be excluded

$\alpha N\beta N$ member of the integrin family, α -DG alpha-dystroglycan, Axl receptor tyrosine kinase, DC-SIGN dendritic cell-specific intercellular adhesion molecule-3-grapping non-integrin, DCIR dendritic cell immunoreceptor, G or GP glycoprotein, HSP heparan sulfate proteoglycan, LSIGN liver/lymph node-specific intercellular adhesion molecule-3-grapping non-integrin, NPC1 cholesterol transporter Niemann-Pick 1, Tyro tyrosine-protein kinase receptor, ? proposed platelet receptor based on indirect cell studies

Table 3 Virus–platelet interactions with moderate hemostatic effects

Virus	TCP	HF	Family	Genome	Receptor on Platelet	Virus Receptor	Other Cell Receptor	Platelet Entry
Rotavirus (Coulson et al. 1997, Fleming et al. 2011)	No	No	Reoviridae	RNA, non-enveloped	$\alpha 2\beta 1$ sialic acid (?)	VP4	$\alpha 2\beta 1$, sialic acid	ND
Coxsackie (Bergelson et al. 1997)	No	No	Picornaviridae	RNA, non-enveloped	CAR	VP1	CAR $\alpha 5\beta 3$, $\alpha 5\beta 5$	ND
encephalomyocarditis virus (Koupenova et al. 2014)	No	No	Picornaviridae	RNA, non-enveloped	TLR7			Yes
Echovirus (Triantafilou et al. 2000, Triantafilou and Triantafilou 2001)	No	No	Picornaviridae	RNA, non-enveloped	$\alpha 2\beta 1$ (?)		$\alpha 2\beta 1$ $\alpha 5\beta 3$	ND
Parechovirus (Triantafilou et al. 2000, Triantafilou & Triantafilou 2001)	No	No	Picornaviridae	RNA, non-enveloped	$\alpha 5\beta 1$ (?)	VP1	$\alpha 5\beta 3$, $\alpha 5\beta 1$	ND

Mostly from the Picornavirus family, certain viruses have been reported to interact with platelets without inducing thrombocytopenia (*TCP*) or hemorrhagic fever (*HF*). These interactions may be indicative of the role of platelets in the innate immune response $\alpha N\beta N$ member of the integrin family, *CAR* coxsackie-adenovirus receptor, *ND* data not available in the literature, *TLR* Toll-like receptor, *VP* virus protein, ? proposed platelet receptor based on indirect cell studies

question, platelets have that binding capacity. The second step in the virus lifecycle is cell entry. Two general mechanisms of cell entry can be initiated upon contact between an animal virus and a target host cell. Most viruses traverse the plasma membrane using the endocytic machinery intrinsic to the cell, whereby the virus is engulfed by a vesicular structure for intracellular transport (Ghigo 2010). Within these endosomal compartments, low pH typically induces the release of genetic contents from the viral nucleocapsid. Based on microscopic morphology and biochemistry, nearly a dozen viral endocytosis mechanisms have been discerned (Cossart and Helenius 2014; Mercer et al. 2010). A second entry mechanism is used exclusively by enveloped viruses. After receptor-mediated cell surface binding, both virus- and host-encoded (Derry et al. 2007) proteins on the virus envelope can engage to form a conformational fusion complex that merges the envelope and plasma membrane.

In contrast to the known interactions between viruses and platelets, little evidence of platelet entry has been documented. Immunogold electron microscopy revealed that purified HIV is trapped within platelet endosomal structures (Youssefian et al. 2002). Another entry mechanism was suggested by colocalization of purified adenovirus with the surface-connected channels of the platelet open canalicular system (Stone et al. 2007). Purified IAV and platelets enabled observation by electron microscopy of the surface association and uptake of virus-like particles into vacuolar structures (Danon

et al. 1959). Furthermore, virus-like particles have been reported in the platelets of DENV-infected patients, although these difficult experiments lacked confirmation of virus (Noisakran et al. 2009). Electron microscopy has also been used to demonstrate encephalomyocarditis virus (EMCV) uptake by platelets in a murine model of infection (Koupenova et al. 2014). To confirm their endocytic capabilities, purified platelets have been shown to engulf *Staphylococcus aureus* (Youssefian et al. 2002) and liposomes that were engineered for drug delivery (Chan et al. 2015). Although viral entry mechanisms are not known, molecular details following the transfer of synthetic particles into platelets suggests that several simultaneous endocytotic pathways are involved (Chan et al. 2015). These pathways may have dynamin dependence as a common aspect and involve caveolae- or clathrin-mediated uptake (Mercer et al. 2010). Thus, observations of virus uptake by platelets are supported by the availability of entry mechanisms for other particle types.

Viral Protein Synthesis Platelets associate with both DNA- and RNA-containing viruses. Assuming successful entry and release of genetic material into the platelet, only RNA viruses replicate because platelets are DNA transcription incompetent. The list of RNA viruses with known or suspected platelet interactions is extensive and highlighted in Tables 1, 2, and 3. Platelets contain all of the post-transcriptional apparatus necessary for potential assembly

of an infectious RNA virus. To demonstrate that platelets can translate viral RNA, purified platelets inoculated with purified DENV have been shown to produce viral nonstructural protein 1 (NS1), as detected by Western blot analysis (Simon et al. 2015). Because the DENV single-stranded RNA (ssRNA) must first be translated as a polyprotein, the finding that NS1 had the predicted molecular weight of the mature protein implied that the functional DENV protease complex (NS2B/NS3) was also properly translated and processed by the platelets (Simon et al. 2015). Thus, platelets not only facilitate the initial step in the virus lifecycle, specific surface engagement (Tables 1, 2, and 3), but also have the means to allow penetration of the virus into the cytoplasm and occupation of the platelet translational mechanism.

Virus Replication Replication of viral genomic material has been followed as a surrogate for functional virus-encoded polymerase generation. In these studies, the mRNAs of all four serotypes of purified DENV in combination with purified platelets were enhanced (Simon et al. 2015), which substantiated an earlier preliminary report (Onlamoon et al. 2010). These studies were extended using virus plaque formation assays in combination with a translation inhibitor and demonstrated that the virus is properly assembled, resulting in production of infectious progeny by platelets (Simon et al. 2015). Interestingly, platelet units stored under blood bank operating conditions also produced new DENV (Sutherland et al. 2016). A generalized model is presented in Fig. 1, highlighting the emerging concept that platelets could be a reservoir for permissive RNA viruses. The concept is based on (1) significant literature demonstrating that many types of virus bind directly to platelets; (2) several studies showing platelet entry; and (3) generation of infectious DENV progeny by platelets.

Although evidence suggests that platelets could be recruited by RNA viruses as conspirators for replication, it is conceivable that only platelet subsets can fulfill this role. As an example, ~20 % of platelets are positive for DC-SIGN (Hottz et al. 2013b) and may account for binding to HIV, DENV, HCV, Ebola, and LASV, which are known to use DC-SIGN for cell surface attachment (Tables 1, 2, and 3). Furthermore, TLR2 is found on a subset of ~14 % of platelets and is involved in at least the CMV interaction (Boehme et al. 2006). Some platelet-interacting viruses are known to use co-receptor systems, of which candidate receptors are found on platelets. But, whether these too are distributed as subpopulations, like DC-SIGN and TLR2, has not been evaluated. Of these functionally distinct classes of platelets, only some may be permissive to entry and replication after binding.

Platelet Immune Response Against Viruses

Platelets may initially harbor and replicate certain viruses (Simon et al. 2015). But, as known facilitators and modulators of the immune response to pathogen invasion (Jenne and Kubes 2015), platelets also competently present bound viruses to leukocytes for clearance. To enhance this innate immune recognition, the association of viruses with platelets induces the release of cytokines, which causes local infiltration of immune cells. Many platelet surface molecules mediate leukocyte cross-talk and have an essential effect on viral infection. The modulation of platelet function by viruses, leading to their ultimate involvement in innate and adaptive immunity, is summarized in Fig. 2.

Toll-Like Receptors TLRs (Cognasse et al. 2015) are a family of innate immune regulators that recognize pathogen-associated molecular patterns (PAMPs), which are markers associated with viruses, bacteria, and fungi that lead to neutrophil-mediated pathogen destruction. Known to recognize ssRNA, typical of many platelet-interacting viruses (Tables 1, 2, and 3), platelet TLR7 was identified as vital for EMCV clearance by platelets (Koupenova et al. 2014). Interestingly, this receptor is expressed within endosomes and its functional involvement implies endocytosis of the virus by the platelet. Penetration of EMCV was TLR7 dependent, with activation of TLR7 resulting in the release of the α -granules that house proinflammatory cytokines, leading to neutrophil aggregation, endothelial cell adhesion, and inflammation. The TLR7 platelet response was shown to contribute to host survival, as EMCV levels decreased and there were no observable prothrombotic events as a consequence of potential platelet activation (Koupenova et al. 2014).

Unlike TLR7, TLR2 is expressed on the platelet surface. CMV was found to associate predominately with the TLR2-positive platelet subpopulation (Boehme et al. 2006). As seen using flow cytometry, CMV induces rapid surface expression of P-selectin, leading to the release of proinflammatory CD40 ligand and interleukin-1 β cytokine from the platelets (Assinger et al. 2014). CMV-induced TLR2 activation was confirmed by antigenic inhibition and could be blocked by inhibiting phosphoinositide 3-kinase signaling. Vascular endothelial-derived growth factor (VEGF) is a proangiogenic cytokine that is released by TLR2-induced platelet activation and involved in endothelial migration, proliferation, and increased vascular permeability (Assinger et al. 2014). To add further to the many links reported between CMV and vascular disease (Al-Ghamdi 2012; Bruggeman 2000), this TLR2-mediated VEGF release can allow leukocyte recruitment to an atherosclerotic plaque, promoting growth (Holm et al. 2009).

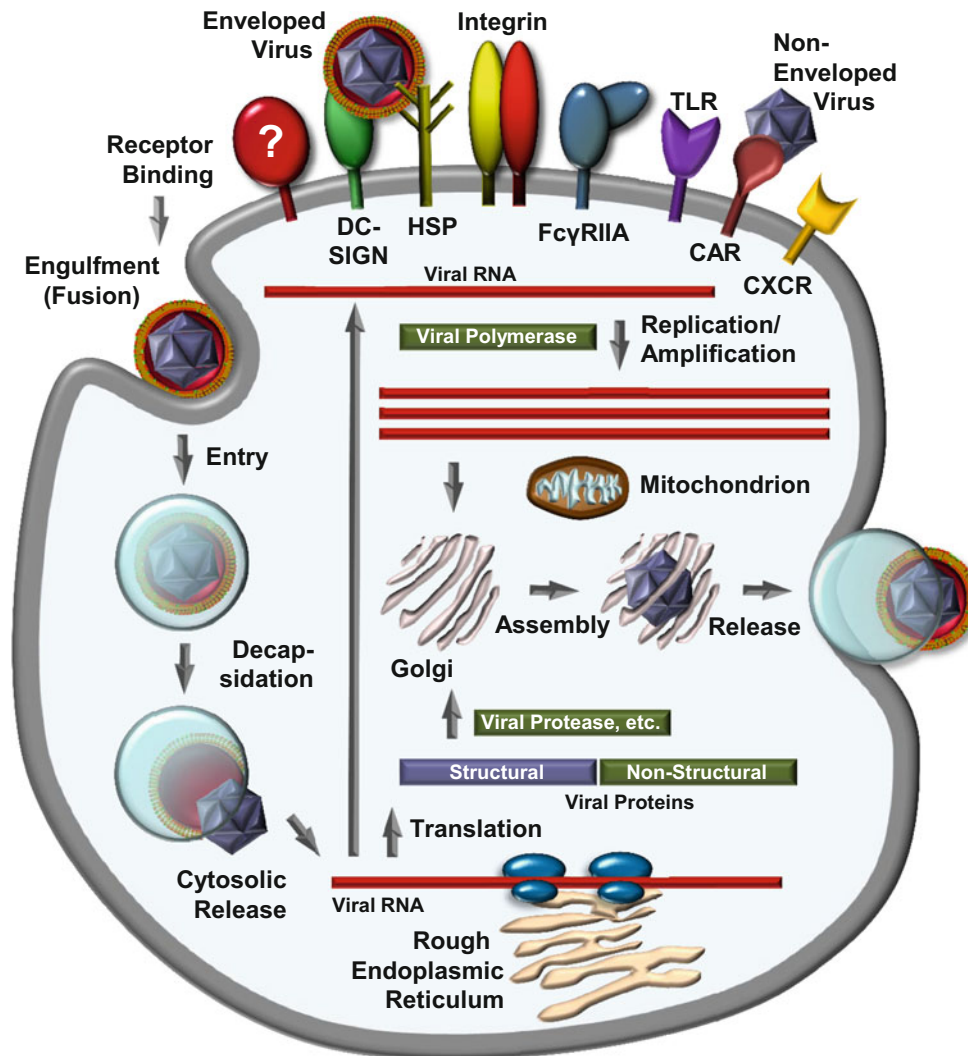


Fig. 1 Virus replication by platelets. Examples of virus receptors present on the surface of the platelet are shown spanning the platelet membrane. Examples of an enveloped virus (DENV binding to the co-receptors DC-SIGN and HSP) and a non-enveloped virus (adenovirus binding to CAR) are depicted. This receptor engagement triggers mechanisms that can involve dynamin-dependent engulfment processes, leading to entry into the platelet as an endosomal inclusion. At least for DENV, which has an RNA genome unlike adenoviruses, evidence is accumulating that suggests viral genomic material is released from the nucleocapsid into the cytoplasm, where it is

translated by platelet ribosomal complexes or used as a template for replication by the virus-encoded polymerase. Other virus-encoded genes contribute to controlling the function of the cell or post-translational modification of the viral proteins, including proteolytic maturation if the viral genome is organized to produce a polyprotein. The viral structural proteins and genome copies are then transported to the platelet Golgi apparatus, where they are assembled and delivered to the exterior by exosomal transport or possibly by cell disruption (not shown). Platelet mitochondria are available for energy-demanding aspects of the mechanism

Integrins Consisting of a broad group of homologous heterodimeric proteins (Bennett et al. 2009), cell surface integrins are fundamental to the important platelet–leukocyte connection. In addition to the role of integrins in facilitating numerous direct platelet–virus interactions (Tables 1, 2, and 3), they are also important in processes leading to immune clearance of blood-borne viruses. One example is through the recruitment of dendritic cells by the interaction of platelet surface junctional adhesion molecule-C (JAM-C) and dendritic cell integrin $\alpha_M\beta_2$ (Langer et al. 2007). Trafficking of cytotoxic T lymphocytes (CTLs) to sites of infection can be mediated by

platelet activation (Iannacone et al. 2005). In the case of hepatitis B virus (HBV), CTLs enter the liver parenchyma and accumulate where HBV may reside and replicate. CTL activity lowers the virus count, but unfortunately contributes to progression of liver damage (Iannacone et al. 2005).

The HIV-1 transactivator of transcription protein (Tat) directly interacts with platelets, resulting in their activation and degranulation (Wang et al. 2011). This mechanism requires the β_3 integrin and chemokine receptor CCR3 to be expressed on the platelet surface. This involvement of integrins was unambiguously demonstrated using a β_3

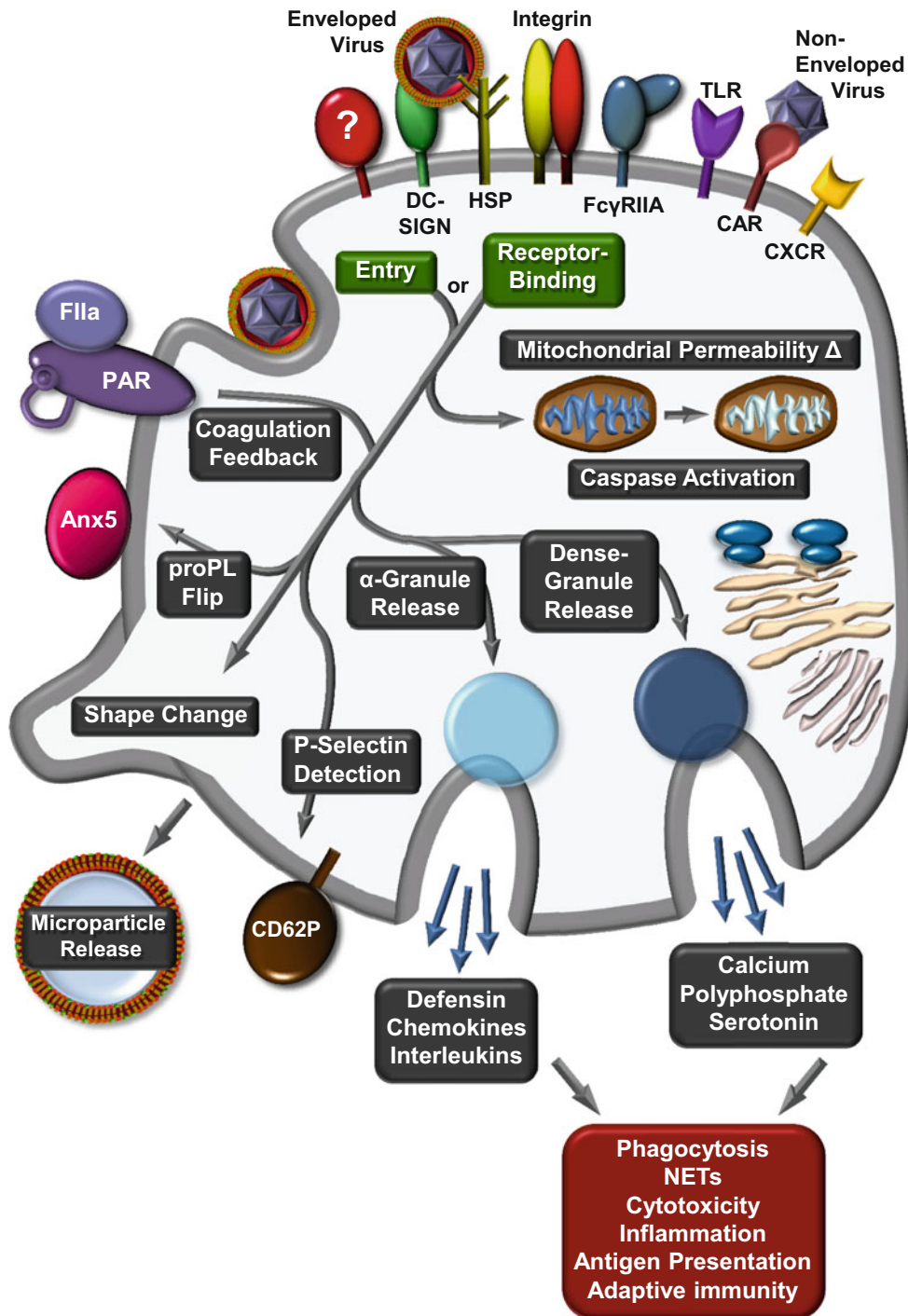


Fig. 2 Virus-induced platelet modulation leading to immune clearance. Platelet receptor binding or possibly entry of the virus induces outside-in platelet signaling, with morphological and biochemical effects to the platelet. Typical platelet “activation” markers are expressed, such as exposure of procoagulant phospholipid (proPL) as measured by annexin V (Anx5) binding and P-selectin (CD62P). Platelet microparticles may be released. The proPL surface can propagate the hypercoagulable state often induced by some of these viruses by enabling assembly of clotting enzyme complexes, where neighboring platelet surface PARs can be activated by thrombin (FIIa) in a

feedback-amplified manner to further promote platelet modulation. Virus-platelet engagement has been shown to induce caspase activation and mitochondrial membrane potential changes indicative of an apoptotic state, which similarly occurs for megakaryocytes (not shown) with consequent reduction in thrombopoiesis. Virus-induced platelet stimulation causes the release of α-granule and dense-granule contents. This has profound stimulatory effects on all cells of the immune system, orchestrating localized innate and adaptive responses against virus invasion

knockout mouse model (Wang et al. 2011). Virus-induced platelet degranulation involves the release of proinflammatory CD40 ligand, which promotes platelet–monocyte complex formation via platelet P-selectin and monocyte P-selectin glycoprotein ligand-1 (PSGL-1) (Singh et al. 2014). This was demonstrated by injecting wild-type mice with recombinant soluble CD40 ligand and analyzing cell associations by flow cytometry. The platelet–monocyte complexes derived from HIV-1 infected donors exhibited enhanced adhesion to human brain microvascular endothelial cells, suggesting a role for platelets in HIV-associated neuroinflammation (Singh et al. 2014).

Selectins Although not as extensive as integrins, selectins are also a family of cellular adhesion receptors. Selectins recognize various carbohydrate structures found on opposing surfaces. Platelets constitutively store P-selectin (i.e., CD62P) in α -granules. In response to stimulus-induced activation, P-selectin is transported to the platelet surface where it mediates tethering to numerous cell types. Important to the role of platelets in orchestrating the immune response, platelet surface P-selectin mediates adhesion through association with PSGL-1 found on the neutrophil surface. P-selectin on platelets also interacts with PSGL-1 on a subset of Th1 leukocytes. Thus, when binding of viruses to the platelet surface triggers platelet activation and P-selectin expression on the surface, innate immune clearance of the pathogen is facilitated.

Protease-Activated Receptors Hemostasis and inflammation are regulated and linked by protease-activated receptors (PARs) (Rothmeier and Ruf 2012). Four PAR types have been identified and are implicated both in virus replication (Aerts et al. 2013; Khoufache et al. 2013; Sutherland et al. 2012) and in the host innate response to viral infection (Antoniak et al. 2013). Enveloped viruses can assemble and activate coagulation enzyme complexes directly on their surface, which mediate PAR stimulation. Through these pathways, PAR1 and PAR2 on human umbilical vein endothelial cells have been shown to enhance HSV1 infection in vitro (Pryzdial et al. 2014). A similar mechanism may stimulate virus-bound platelets, which express high levels of PAR1. A more recent study showed that IAV induced platelet activation and aggregation through platelet-surface PAR, which exacerbated acute lung injury (Le et al. 2015). This injury was attributed to the resulting massive inflammation through platelet-induced recruitment of neutrophils to the lung.

Chemokine Receptors Chemokine receptors are members of the G-protein-coupled receptor family, whose major functions include cellular recruitment via chemokine recognition. Several chemokine receptors (CCR1, CCR3, CCR4, CXCR1, and CXCR4) bind to select ligands, resulting in

enhancement but not initiation of inflammatory pathways, platelet aggregation, hemostasis, and thrombosis (Zarbock et al. 2007). Activated platelets also secrete numerous chemokines, such as CXCL7 and CXCL8. CXCL7 promotes chemotaxis, adhesion to endothelial cells, and degranulation of neutrophils (Schenk et al. 2002), whereas CXCL8 is important in recruitment of neutrophils (Baggiolini et al. 1994). Active CD40L is secreted by platelets in response to stimulation and binds endothelial CD40, eliciting chemokine secretion and increasing the expression of adhesion molecules on the endothelium (Henn et al. 1998).

Platelet α -granule contents have been shown to limit the spread of HIV-1 in co-cultured T cells (Solomon et al. 2013). CXCL4 (platelet activating factor) and CXCL7 are the most abundant chemokines in the α -granules of platelets (Blair and Flaumenhaft 2009). In particular, CXCL4 released by activated platelets binds to HIV-1 major viral envelope glycoprotein, gp120, proximal to the essential CD4-binding site (Auerbach et al. 2012). The resulting steric inhibition reduced HIV-1 infection by 80 % compared with non-activated platelets using a HIV-1-sensitive cell line that uses reporter gene constructs to quantify infection (Solomon et al. 2013). CXCL4 stimulates neutrophil–endothelial cell attachment and also acts as a co-stimulator of TNF in the release of neutrophil secondary granules (Kasper and Petersen 2011), as further means of enhancing localized immune cell influx.

The proinflammatory cytokine interleukin (IL)-1 β is synthesized by platelets as a precursor protein and cleaved by caspase-1 to produce an active form that is released in microparticles (Hottz et al. 2013b). DENV2 was shown to induce IL-1 β synthesis directly and secretion from platelets by activating the assembly of a nucleotide-binding domain leucine-rich repeat-containing protein (NLRP3) inflammasome, which controls caspase-1 activity (Hottz et al. 2013a). Generally, IL-1 β is important in the acute-phase response, where proteins such as C-reactive protein, complement components, and fibrinogen are produced to destroy or contain microbes (Morrell et al. 2014). Although aiding in the immune response, the IL-1 β released from platelets during DENV infection contributes to increased endothelial permeability, thrombosis, and dysregulated hemostasis (Hottz et al. 2013a).

Defensins Defensins are cationic antimicrobial peptides that are key elements in the innate immune system. They act on bacteria, enveloped viruses, and non-enveloped viruses. There are many forms of these small 4–5 kDa peptides, with human platelets expressing β -defensins (hBD) 1, 2, and 3 (Kraemer et al. 2011; Tohidnezhad et al. 2011; Tohidnezhad et al. 2012). Immunofluorescence studies in vitro showed that the inclusion of a selective agonist of neutrophils (phorbol 12-myristate 13-acetate) or platelet PAR1 (thrombin receptor agonist peptide 1) induced

secretion of an adhesive complex from neutrophils, identified as a pathogen “snare.” The molecular networks were identified as being composed of long uncoiled strands of DNA and were named neutrophil extracellular traps (NETs) (Brinkmann et al. 2004). NET release is induced by β -defensin 1 secreted from activated platelets (Kraemer et al. 2011). Although most NET involvement in pathogen defense has been elucidated using bacteria, super-resolution structured illumination microscopy revealed that HIV-1 virus particles are also captured by NETs (Saitoh et al. 2012). When these entrapped virus particles were extracted, their infectivity was reduced as a result of highly enriched levels of α -defensin and myeloperoxidase within the NETs.

In addition to HIV-1, hBD-2 is also known to inhibit human respiratory syncytial virus (RSV) entry into human lung epithelial cells, as followed by ^{35}S -labeled RSV uptake (Kota et al. 2008). It was also shown through electron microscopy and buoyant density profiles that hBD-2, but not hBD-1, disrupted the viral envelope, possibly because the cationic nature of the peptide led to lipid destabilization (Kota et al. 2008). HIV-1 induced hBD-2 and hBD-3 expression in human oral epithelial cells, which associated directly with HIV-1 and neutralized infection in vitro (Quinones-Mateu et al. 2003). IAV hemagglutinin and hBD-3 binding also resulted in inhibition of epithelial cell infection (Leikina et al. 2005). hBD-3 was shown to alter fusion between IAV, sindbis virus, baculovirus, and synthetic target membrane by crosslinking virus surface proteins (Leikina et al. 2005). Although reasonable to speculate, it is not known whether platelet-derived hBD-2 and hBD-3 mediate similar direct antiviral effects.

Other Secreted Platelet Components Human donor platelet concentrates contain unknown antiviral activity that reduced the viral titer of poliovirus 1, adenovirus 5, and vaccinia virus by approximately 2.63 ± 0.5 to 5.6 ± 0.9 log units (Maurice et al. 2002). The same group also observed platelet activation in all virus–platelet co-cultures with epithelial monolayers (Vero cells); recent knowledge (Flaujac et al. 2010) suggests that platelet releasate is the antimicrobial factor(s) in these studies.

Defensins and cytokines are stored in platelet α -granules. However, dense granule constituents such as adenosine diphosphate (ADP) (Packham and Rand 2011), polyphosphates (polyP) (Smith and Morrissey 2014), and serotonin (Jedlitschky et al. 2012) also have immunomodulating properties (Morrell et al. 2014). These have not been as well characterized for a role in viral innate immunity as those contained in α -granules. Nevertheless, the release of platelet dense granule contents by viruses has been investigated in CMV infection. This was mediated by platelet surface TLR2 occupation. Furthermore, inhibition of the

ADP receptor, P2Y₁₂, identified ADP release as an important trigger for secondary platelet activation (Assinger et al. 2014). Dendritic cells also express P2Y₁₂ and their stimulation increases antigen endocytosis and processing (Vanderstocken et al. 2010), which could involve localized platelet response to pathogen interactions. PolyP induces proinflammatory responses by acting on the nuclear factor κB (NF- κB) pathway in vascular endothelial cells (Bae et al. 2012). PolyP can also interact with chromatin-associated nuclear proteins such as high mobility group box 1 (HMGB1) to amplify proinflammatory responses (Dinarvand et al. 2014). Because HMGB1 is also secreted by platelets (Maugeri et al. 2012), polyP warrants continued study as a bridge between platelets and innate immunity, especially in the context of complement, which is suppressed by polyP (Wat et al. 2014) and could be a viral survival mechanism.

Virus-Induced Thrombocytopenia

According to some estimates, approximately two-thirds of acute thrombocytopenia cases are preceded by viral infection (Rand and Wright 1998). This strikingly high correlation suggests mechanisms directly linking the virus to the platelet, such as receptor-mediated binding (Table 1). In contrast to viral thrombocytopenia, viral HF (Table 2) is thought to be driven by severe suppression of innate immunity and the resulting cytokine flood that counters systemic virus replication. HF can also result in a reduced platelet count, but through mechanisms indirectly involving platelets, as detailed in excellent reviews elsewhere (Feldmann and Geisbert 2011; Messaoudi and Basler 2015; Zapata et al. 2014). Here, we overview five primary pathways that simultaneously contribute to viral thrombocytopenia in the absence of HF: decreased thrombopoiesis, direct and indirect virus interactions with platelets and megakaryocytes (tipping the intricate balance between thrombopoiesis and platelet clearance), altered platelet function, and virus-induced immune response against platelets.

Decreased Thrombopoiesis Viral infection results in production of interferon, which has antiviral activity. However, type I interferons can also inhibit megakaryocytes, resulting in impaired platelet production (Rivadeneira et al. 2015). Megakaryocyte growth and differentiation is stimulated by thrombopoietin (TPO), which is predominantly produced in the liver (Giannini et al. 2002). Impaired liver function is consequently detrimental to TPO production and the resulting thrombocytopenia often suffered by chronic HCV patients correlates with low TPO and attenuated thrombopoiesis (Giannini et al. 2002; Wenzel et al. 2010).

Direct impairment of platelet production as a result of viral replication in megakaryocytes was observed *in vitro* for HIV, HCV, DENV, and CMV (Basu et al. 2008; Crapnell et al. 2000; Li et al. 1999; Sato et al. 2000; Sridharan et al. 2013). An increase in cell death via apoptosis and decreased megakaryocyte differentiation are probable reasons for reduced platelet production. For example, HIV infection of megakaryocytes resulted in downregulation of the TPO receptor c-Mpl (Gibellini et al. 2013), causing reduced sensitivity to thrombopoiesis induction. To further investigate this mechanism, umbilical cord blood hematopoietic progenitor cells that were induced by TPO toward a megakaryocytic lineage *in vitro* have been used as a model for HIV infection. The virus surface glycoprotein gp120 interacts with CD4 on these cells (Gibellini et al. 2007), resulting in specific protein and mRNA changes that elevate TGF- β 1 levels and decrease tumor necrosis factor (ligand) superfamily, member 13 (TNFSF13) levels. Both of these effects contribute to inhibition of megakaryocytic proliferation and promote apoptosis (Gibellini et al. 2007). Further evidence supporting the concept that viral thrombocytopenia involves attenuated thrombopoiesis comes from an elegant humanized mouse model showing that DENV infection reduces platelet production (Sridharan et al. 2013). Thus, viral infection has the capacity to reduce thrombopoiesis by direct effects on the megakaryocyte, conceivably involving the same surface receptors for specific viruses that have been identified on platelets and other cells (Tables 1, 2, and 3).

Direct Virus–Platelet Associations Activation of platelets, degranulation, and recruitment of neutrophils and dendritic cells contribute to phagocytosis of platelet fragments by leukocytes. The final clearance of these potentially virus-laden particles is in the spleen and liver (Bondanza et al. 2001; Grozovsky et al. 2010; Koupenova et al. 2014). CMV- or EMCV-mediated activation of platelets via TLR2 or TLR7, respectively, enhanced the interaction between platelets and neutrophils, resulting in platelet clearance (Assinger et al. 2014; Koupenova et al. 2014). In a similar manner, hantavirus also induced platelet activation and clearance by binding to platelet surface integrins $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ (Gavrilovskaya et al. 2010). The interaction of hantavirus with platelets also contributed to viral dissemination and activation of endothelial cell functions, thereby increasing vascular permeability (Feldmann and Geisbert 2011; Gavrilovskaya et al. 2010).

Adenovirus correspondingly induces thrombocytopenia, as seen when adenovirus gene therapy vectors are intravenously administered to rhesus macaques and mice (Othman et al. 2007; Wolins et al. 2003). Current literature indicates that CAR mediates the binding of adenovirus to platelets, enabling subsequent entry (Othman et al. 2007; Shimony et al. 2009). The virus–platelet interaction is predominantly

localized to sites of intercellular complex formation, implying that CAR expression is enhanced in response to platelet activation (Gupalo et al. 2011). Similar to the effects reported for other viruses, platelet activation is probable upon the initial association of adenovirus and platelet-surface CAR.

The IAV envelope has neuraminidase activity that cleaves sialic acid on the surface of platelets (Madoff et al. 1964). Removal of more than 15 % of total sialic acid on rabbit platelets caused complete platelet clearance within 1 h of administering ^{51}Cr -labeled platelets into rabbits (Greenberg et al. 1975). The removal of circulating platelets was presumably caused by recognition of exposed terminal galactose residues by scavenger cells expressing the asialoglycoprotein receptor (Sorensen et al. 2009). This adds to the repertoire of virus-clearance mechanisms that can be facilitated through direct virus–platelet association.

Indirect Virus–Platelet Associations Platelets express Fc receptors that allow recognition of immune complexes or aggregated immunoglobulin. In addition to PAR-1 activation by thrombin, IAV can activate platelets through low-affinity Fc γ RIIA signaling (Boilard et al. 2014). For this to occur, IAV must be decorated with anti-IAV IgG. Crossreactive antibodies resulting from immune recognition of different IAV strains (H1N1 versus H3N2) was sufficient to produce this effect. When wild-type mice (which do not express Fc γ RIIA) and transgenic mice expressing human Fc γ RIIA were challenged intravenously with a sublethal dose of H1N1, there was a drop in circulating platelet count in only the transgenic mice. This indicates that platelet homeostasis is affected by the accessibility of Fc receptors on platelets, supporting the link to pathogenic thrombocytopenia (Boilard et al. 2014).

Activation of endothelial cells has been implicated in adenovirus-induced thrombocytopenia. In these studies, the release of ultralarge von Willebrand factor (vWF) from the Weibel–Palade bodies (Gupalo et al. 2011) was evaluated using a murine model. Adenovirus induced thrombocytopenia in wild-type animals but, in sharp contrast, vWF knock-out mice were protected from a reduced platelet count (Gupalo et al. 2011). Thus, endothelial vWF could contribute in an indirect manner to virus-induced thrombocytopenia by supporting platelet aggregation through interactions with platelet surface GP1b, (the vWF receptor). Clearance of these platelet aggregates is subsequently facilitated by splenic macrophages or Kupffer cells in the liver (Othman et al. 2007).

Systemic inflammation as a result of viral infection can result in platelet interactions and increased clearance, as demonstrated during IAV, rhinovirus, and CMV infections (Bouwman et al. 2002). The mechanism involves mononuclear leukocytic release of CXCL4. DENV infection was

used in a subsequent study and resulted in increased inflammation, vascular permeability, and platelet aggregation and activation (Yang et al. 1995).

Altered Platelet Function Thrombocytopenia can result from virus activation of platelets or endothelial cells, which induces cell–cell adhesion processes via expression of integrin and selectin function (Zapata et al. 2014). The flip-side is that inhibition of platelet aggregation can also result in impaired platelet function. DENV infections have been shown to stimulate platelet-directed IgM autoantibodies that inhibit ADP-induced platelet aggregation (Lin et al. 2001). Although these autoantibodies have an effect on immune clearance of platelets, they also affect the development of HF. For hantavirus, this can include renal syndrome resulting from defective platelet aggregation (Cosgriff et al. 1991). Other HF viruses such as Lassa virus, Junin virus, and Ebola virus have inhibitors of platelet aggregation, but these have not been identified (Cummins et al. 1989; Cummins et al. 1990, Feldmann and Klenk 1996). Additionally, vaccinia virus causes impairment of platelet aggregation induced by ADP, collagen, or thrombin (Bik et al. 1982). Ebola virus infection also results in increased levels of type I interferons, which downregulate platelet production and function (Rivadeneira et al. 2015; Villinger et al. 1999).

Virus-Induced Immune Response Against Platelets The molecular relationship between autoantibodies against platelet proteins and viral infection is complex and not yet clearly understood. Virus-induced thrombocytopenia typically worsens as damage to the liver progresses and can deteriorate into a more severe clinical complication (Aref et al. 2009). Anti-platelet autoantibodies are linked to immune thrombocytopenic purpura (ITP) (Liebman 2008). Secondary ITP can result from vaccines such as measles-mumps-rubella (MMR) (incidence of 1 in 40,000 administrations) or infections with homologous herpes family viruses, hepatitis C, HIV, hantavirus, and severe acute respiratory syndrome coronavirus (Goeijenbier et al. 2012; Liebman 2008).

Non-AIDS early HIV-1 infections can result in ITP induced by autoimmune antibodies. Affinity purification of circulating serum immune complexes in HIV patients identified an anti-HIV IgG1 antibody that recognizes amino acid residues 49–66 of the integrin $\beta 3$ subunit that induces platelet vesiculation (Nardi et al. 2001). The complement pathway was ruled out in this reaction because neither the $F(ab')_2$ fragment of the antibody raised against integrin $\beta 3$ residues 49–66 in wild-type mice nor treatment with full-length antibody in C3-deficient mice could affect platelet microparticle formation (Nardi et al. 2001). Peroxide generation was monitored through the use of an intracellular fluorescent probe and revealed a novel mechanism by

which the autoantibody induced damage in platelets through a NADPH oxidase peroxide-generating pathway (Nardi et al. 2001). Similar outside-in signaling events could be generated by other virus-induced platelet autoantibodies.

ITP occurs in 20 % of HCV patients and is potentially attributed to the presence of antibodies that are crossreactive with HCV core envelope 1 protein and platelet $\beta 3$ integrin (Rajan et al. 2005; Zhang et al. 2009). The crossreactivity was found by using the antibody specific for integrin $\beta 3$ residues 49–66 to screen a phage-display peptide library (Zhang et al. 2009). The recognized peptides were then aligned with the viral genome to define similarities. The matched peptides were rationally designed as tools to inhibit the binding of autoantibodies to platelets or to produce platelet antibodies with functional effects (Li et al. 2005; Zhang et al. 2009). This approach was similarly used to discover molecular mimics in the HIV-1-encoded negative regulatory factor (nef) protein (Li et al. 2005), both leading toward therapeutic design in virus-induced thrombocytopenia.

Antibody crossreactivity between antiviral antigens and platelet antigens has been demonstrated during DENV infection (Cheng et al. 2009). Pairwise sequence alignment analysis programs were used to annotate homologous peptide sequences between DENV NS1 and platelet protein disulfide isomerase (PDI) (Cheng et al. 2009). This tool identified several sequence homologies, of which amino acid residues 311–330 (P311–330) was the most dominant epitope recognized by both anti-NS1 and anti-PDI, as determined by ELISA (Cheng et al. 2009). The P311–330 antibodies generated from hyperimmunized mice bound platelet PDI and inhibited both thiol isomerase activity and platelet aggregation induced by ADP (Cheng et al. 2009). The presence of anti-platelet autoantibodies with anti-DENV activity in DENV patient sera is associated with thrombocytopenia and the severity of the disease during the acute phase of secondary DENV infection (Saito et al. 2004). However, the direct implication of these crossreactive antibodies in DENV pathogenesis has not yet been elucidated. The discovery that platelets can translate and express NS1 (Simon et al. 2015) suggests that viral antibodies might not be crossreactive with platelets, but actually recognize the virus-encoded gene product expressed on the platelet, further complicating vaccine development.

Conclusion

Associations between viruses and platelets can lead to pathology. Viral infection often precedes thrombocytopenia and, therefore, an understanding of the mechanisms that facilitate virus–platelet interactions, their direct effect on platelets, and indirect effects on the cellular environment can lead to therapeutic control. This is a difficult challenge because platelets also help to eradicate viruses by steering

innate and adaptive immune responses. Thus, ideal therapeutic control of virus-induced thrombocytopenia can discretely manage both the detrimental and positive involvement of platelets in viral infection.

Take Home Messages

- Diverse virus families can bind to platelets, resulting in mild to severe clinical outcomes.
- Virus–platelet interplay results in changes to innate and adaptive immunity.
- Platelets replicate the RNA genome of permissive viruses, which can contribute to pathology.

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