Cellular & Molecular Immunology (2015) 12, 264–268

www.nature.com/cmi

MINI REVIEW

Chronic hepatitis B: role of anti-platelet therapy in inflammation control

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Platelets play a known role in the maintenance of vascular homeostasis, but these cells are emerging as important cellular mediators of acute and chronic inflammatory diseases. Platelets are key elements in the pathogenesis of acute and chronic liver disease associated with hepatitis B virus (HBV) infection by promoting the accumulation of virus-specific $CD8^+$ T cells and nonspecific inflammatory cells into the liver parenchyma. This review discusses major platelet functions in immune and inflammatory responses, with an emphasis on recent pre-clinical studies that suggest that the inhibition of platelet activation pathways represent an alternative therapeutic strategy with potential use in the reduction of virus-specific T cell-mediated chronic inflammation, liver fibrosis and hepatocellular carcinoma in patients who are chronically infected with HBV.
Cellular & Molecular Immunology (2015) **12,** 264–268; doi:10.1038/cmi.2014.124; published online 12 January 2015

Keywords: anti-platelet drugs; $CD8^+$ T cells; chronic hepatitis B; hepatocellular carcinoma; platelets

PLATELETS IN IMMUNITY AND INFLAMMATION

Platelets are small, disk-shaped, anucleated cellular fragments (diameter≈2 μM and ≈0.5 μM in humans and mice, respectively) that are released into circulation by megakaryocytes, bone marrow-resident platelet precursors.[1] During their lifespan (~8 and ~4 days in humans and mice, respectively),[1] platelets are the chief cellular mediators of hemostasis and thrombosis.[2] Ongoing discoveries highlight the central role of platelets in immunity and inflammation during health and disease. Relevant works that describe platelet contributions in immune functions and different inflammatory and immune-mediated diseases are summarized in Table 1.[3–13] Additionally, platelets are the most abundant cell type with immune function in blood circulation (~200 000/μl and ~900 000/μl in humans and mice, respectively).[1]

Platelet immunological functions are achieved by the expression of a variety of adhesion molecules and immune receptors on their membrane that are instrumental for interactions with the endothelium and different subsets of circulating leukocytes.[14,15] For example, platelet activation induces the mobilization of P-selectin, which mediates interactions with cells that express its ligand, P-selectin glycoprotein ligand-1, such as neutrophils, monocytes and other leukocytes.[16,17] Platelets also express several integrins, including αIIbβ3, α2β1, α5β1 and α6β1, which promote interactions with endothelial cells, extracellular matrix components and leukocyte populations that express integrin ligands, such as intercellular adhesion molecule-1 and junctional adhesion molecules.[18,19]

Platelets also possess a wide array of preformed inflammatory molecules and immune mediators that are stored inside three different intracellular granules, defined as α-, δ- and λ-granules (Table 2).[20] α-granules are the most abundant type, and platelet activation induces the release/exposure of a plethora of mediators on the platelet surface that promote homotypic and heterotypic cellular interactions (e.g., P-selectin), including the activation (e.g., CD40L) and recruitment of inflammatory cells to the site of inflammation (e.g., chemokines) and the expression of proteins that promote cellular proliferation and neo-angiogenesis (e.g., TGF-β, PDGF and VEGF) (Table 2).[21] In addition, platelets store electrondense structures called δ-granules, or dense bodies, that contain nucleotides (e.g., ADP, ATP) (Table 2)[21] that are important to sustain secondary platelet activation and vasoactive molecules, such as histamine and serotonin, which are important for the maintenance of vascular tone (Table 2).[22] Finally, λ-granules (lysosomes) contain proteins with bactericidal properties, including collagenase, elastase and β-glucuronidase (Table 1).[20] The storage of preformed molecules enables their

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Received: 14 July 2014; Revised: 30 October 2014; Accepted: 19 November 2014

Table 1 Platelet contribution to inflammation and immunity

Title	*Disease*	*Molecule(s)*	*Mechanism(s)*
'Platelet-mediated lymphocyte delivery to high endothelial venules'[3]	—	P-selectin	P-selectin promotes WBC rolling on high endothelial venules
'CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells'[4]	—	CD40L	CD40L on platelets induces endothelium to secrete chemokines and express adhesion molecules
'Platelet-mediated modulation of adaptive immunity: a communication link between innate and adaptive immune compartments'[6]	—	CD40L	Platelets, *via* CD154, induce dendritic cell maturation, B-cell isotype switching and augment $CD8^+$ T cell responses
'Platelet P-selectin facilitates atherosclerotic lesion development'[5]	Atherosclerosis	P-selectin	P-selectin enhances and facilitates the formation of atherosclerotic lesions
'Platelets mediate cytotoxic T lymphocyte-induced liver damage'[8]	Viral hepatitis	—	Platelets promote the accumulation of virus-specific $CD8^+$ T cells into the liver
'Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood'[9]	Sepsis	TLR4	TLR4-dependent platelet–neutrophil interaction leads to the production of NETs
'Platelets kill intraerythrocytic malarial parasites and mediate survival to infection'[10]	Malaria	PF4	Platelets release PF4 upon contact with parasitized red cells and it directly kills intraerythrocytic parasites
'Platelets amplify inflammation in arthritis *via* collagen-dependent microparticle production'[11]	Rheumatoid arthritis	GPVI; IL-1	GPVI triggers platelet pro-inflammatory microparticles eliciting cytokine responses from synovial fibroblasts *via* IL-1
'Platelets contribute to allograft rejection through glutamate receptor signaling'[12]	Transplant rejection	Glutamate; TBX-A_2	Platelets contribute to T-cell recruitment and increase plasma inflammatory mediators (i.e., glutamate and TBX-A_2) accelerating T cell-mediated skin graft rejection
'Platelets contribute to the pathogenesis of experimental autoimmune encephalomyelitis'[13]	EAE	GP Ibα; GP IIbIIIa	Platelets promote the recruitment of leukocytes to the inflamed CNS

Abbreviations: CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; GPVI, glycoprotein VI; IL-1, interleukin 1; TBX-A2, thromboxane A_2; TLR4, Toll-like receptor 4; WBC, white blood cells.

Table 2 Platelet granule contents, surface molecules, and platelet-derived mediators involved in inflammation and immunity

	α-granules	*δ-granules*	*Lysosome granules*	*Other factors with immune functions*
Number per platelet	50–80 per platelet	3–8 per platelet	<3	
Contents	**Adhesive glycoproteins** P-selectin, CD40L, fibrinogen, vWF, thrombospondin and fibronectin **Coagulation factors** Factor V, XI, XIII and protein-S **Mitogenic factors** TGF-β, EGF and PDGF **Angiogenic factors** VEGF, and PF4 inhibitor **Fibrinolytic inhibitors** α2-plasmin inhibitor, PAI-1 **Immunoglobulins** **Granule membrane-specific proteins** P-selectin, GMP33 and CD63 **Chemokines** CXCL7, CXCL4 (PF4), CXCL1 (GROα), CXCL5, CCL5 (RANTES), CCL3 (MIP1α)	**Amines** Histamine and serotonin **Bivalent cations** Ca^{2+} and Mg^{2+} **Nucleotides** ATP, ADP, GTP and GDP	**Glycohydrolases** Heparinase, β-N-acetyl-glucosaminidase, β-glucuronidase, β-glycerophosphatase, β-galactosidase, α-D-glucosidase, α-L-fucosidase, β-D-fucosidase **Acid proteases** Carboxypeptidases (A, B), cathepsins D and E, acid phosphatase, collagenase	**Cytoplasmic** CCL7 (MCP3), IL-1β, HMGB1, defensins, TX-A_2, PAF **Membrane associated** TLR1, TLR2, TLR5, TLR4 TLR6, CD40, CD40L, TREM-1 ligand

Abbreviations: EGF, epidermal growth factor; PDGF, platelet-derived growth factor; PAF, platelet activating factor; PAI-1, plasminogen activator inhibitor 1; TGF-β, transforming growth factor β; TLR, Toll-like receptor; TX-A2, thromboxane A_2; VEGF, vascular endothelial growth factor; VWF, von Willebrand factor.

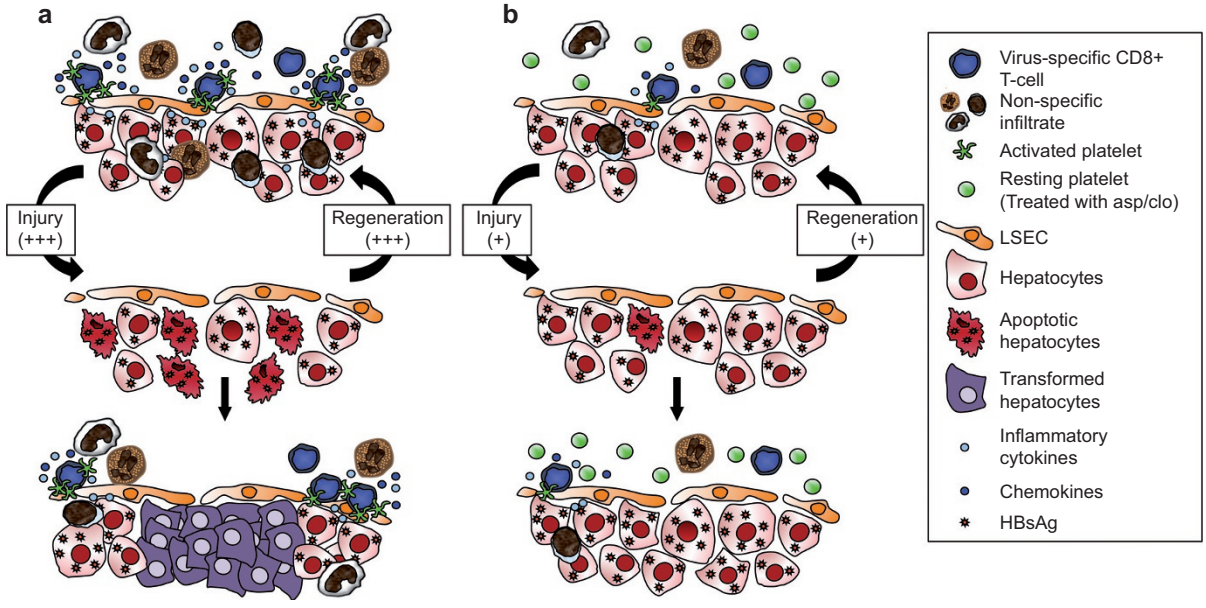

Figure 1 Antiplatelet therapy prevents the development of immune-mediated HBV-induced HCC. (**a**) Activated platelets promote the intrahepatic accumulation of virus-specific CTLs. Upon recognition of infected hepatocytes, virus-specific CTLs produce inflammatory cytokines and induce the intrahepatic expression of chemokines, which recruit additional nonspecific inflammatory cells into the liver parenchyma and promote hepatocellular death. Repetitive cycles of necro-inflammatory liver cell death and regeneration in a mutagenic/mitogenic environment promote the development of HCC. (**b**) Inhibition of platelet activation using dual anti-platelet treatment significantly reduces the number of recruited virus-specific CTLs and secondary nonspecific infiltrates and reduces liver injury, compensatory hepatocyte regeneration and the overall development of HCC. Asp/Clo, aspirin and clopidogrel treated; HBsAg, Hepatitis B virus surface antigen; LSEC, liver sinusoidal endothelia cells.

rapid release upon activation, but platelets also synthesize inflammatory mediators, such as IL-1β, *de novo* (Table 2).[23] In this scenario, platelets play an important role in the timely control of infections and inflammation by interacting with endothelial and immune cells and releasing/producing pro-inflammatory mediators.[10,24,25] However, interactions between platelets and inflammatory cells that are chronically maintained promote an excessive and detrimental inflammatory immune stimulation that may lead to immunopathology and tissue destruction.

PLATELETS IN ACUTE HEPATITIS

In addition to work that identified platelets as important contributors of inflammatory and immune-mediated diseases, our group identified platelets as important players in the pathogenesis of hepatitis B virus (HBV)-related liver disease by sustaining the intrahepatic accumulation of virus-specific T cells, the intrahepatic expression of pro-inflammatory cytokines (e.g., IFN-γ) and chemotactic factors that promote the recruitment of antigen nonspecific inflammatory cells.[8] Specifically, we showed that platelets were detectable inside the liver necro-inflammatory foci that are characteristic of acute liver disease, together with small numbers of virus-specific $CD8^+$ T cells and nonspecific inflammatory cells that contribute to disease pathogenesis using two different models of acute viral hepatitis, HBV-replicating mice injected with HBV-specific $CD8^+$ T cells and wild-type mice infected with an hepatotropic adenovirus.[8] Notably, platelet depletion reduced the intrahepatic accumulation of virus-specific effector $CD8^+$ T cells and nonspecific inflammatory cells that were secondarily recruited and ultimately ameliorated the severity of liver disease.[8]

ANTI-PLATELET THERAPY AMELIORATES ACUTE AND CHRONIC VIRAL HEPATITIS

The exact mechanism by which platelets sustain the recruitment of inflammatory cells into the liver is not known, but we found that two platelet-specific activation inhibitors, aspirin, which blocks thromboxane A_2 production, and clopidogrel, which blocks the $P2Y_{12}$ ADP receptor,[26] reduced the accumulation of virus-specific $CD8^+$ T cells in the liver and the associated liver damage when administered alone or as a combination therapy. These results identified a functional link between platelets and virus-specific $CD8^+$ T cells.[27]

Long-term oral aspirin and clopidogrel are used in patients who are at risk of thrombosis. Therefore, we took advantage of a previously established mouse model of immune-mediated chronic hepatitis B[28] to determine whether the sustained inhibition of platelet activation reduces the severity of chronic liver injury and its life-threatening complications, including cirrhosis and hepatocellular carcinoma (HCC). Chronic hepatitis B is characterized by a functionally inefficient virus-specific $CD8^+$ T-cell response that fails to eliminate HBV from the liver but maintains continuous cycles of low-level hepatocellular death and compensatory hepatocellular proliferation. These cycles are the driving force for the random genetic alterations that occur over several decades in an environment enriched with

inflammatory mutagens, which induces HCC (Figure 1a).[29] Alternative mechanisms of carcinogenesis that are often attributed to HBV, such as HBV DNA integration (inside or in the proximity of genes that control cellular proliferation, survival and differentiation), and the expression of pro-carcinogenic viral products (e.g., HBx and HBs)[30] represent additional factors of hepatocarcinogenesis that are secondary to the chronic inflammation mediated by virus-specific T cells.[29] Notably, the inhibition of platelet activation pathways using clinically achievable doses of the anti-platelet drugs aspirin and clopidogrel, which are continuously administered after the induction of acute hepatitis, reduces the intrahepatic accumulation of virus-specific $CD8^+$ T cells and the secondary recruitment of additional pathogenic antigen nonspecific inflammatory cells, which prevents overall hepatocarcinogenesis and greatly improves survival in this model system (Figure 1b).[31]

Mechanistically, aspirin treatment at clinically relevant doses has no direct anti-inflammatory ability,[26] but it inhibits the release of serotonin and other small molecules from δ-granules. Aspirin has no effect on the release of proteins and peptides stored in α-granules, which are potentially important in the cross-talk between platelets and virus-specific $CD8^+$ T cells.[6,16,24] Conversely, clopidogrel inhibits the expression of α-granule-stored proteins that are involved in heterotypic interactions between platelets/leukocytes and the endothelium, such as P-selectin and CD40L.[32] These observations suggest that the combined use of aspirin and clopidogrel ameliorates the course of immune-mediated chronic hepatitis and HCC progression through distinct pharmacological effects. The synergistic action of these two drugs may represent a new therapeutic strategy to reduce the platelet-dependent accumulation of pathogenic virus-specific $CD8^+$ T cells, and consequently, the accumulation of virus-nonspecific inflammatory cells, hepatocellular injury and compensatory proliferation, liver fibrosis and HCC development.

These results support the hypothesis that a sustained immune-mediated necro-inflammatory liver disease involving platelets is responsible for the development of HCC in an HBV transgenic mouse model. The results also suggest that similar events are responsible for the development of HCC in chronically infected patients.[31,33] Notably, anti-platelet therapy does not appear to act on alternative platelet-derived pro-tumoral factors that are implicated in the pathogenesis of chemically induced HCC.[31] Accordingly, anti-platelet therapy did not reduce PDGF and VEGF [31] or overall HCC development in CCl_4-induced hepatocarcinogenesis, a model in which transformation is independent of adaptive immune responses. These results further sustain the concept that a harmful virus-specific immune response is necessary and sufficient to drive liver cancer during chronic infections with a hepatotropic virus, such as HBV.[31]

CONCLUDING REMARKS

Clinically, anti-platelet therapy may represent a novel fascinating approach for the management of patients who are chronically infected with HBV. We believe that patients in the initial and active phases of the disease (e.g., high ALT) may represent ideal targets who may benefit the most from the long-term daily administration of anti-platelet therapy. Aspirin and clopidogrel reduced the intrahepatic recruitment of virus-specific $CD8^+$ T cells and secondarily recruited antigen nonspecific inflammatory cells, which may negatively impact the control of HBV replication. Therefore, caution must be taken during the administration of these drugs, and HBV viremia must be carefully monitored. In this view, combined aspirin–clopidogrel therapy may firstly be associated with antiviral therapy to control viral replication and reduce overall liver inflammation. An additional concern about this treatment is an increased risk of bleeding in individuals with compromised coagulation associated with impaired liver function. However, limiting treatment to patients who are at a low risk of bleeding during the early stages of chronic disease may be beneficial in reducing thrombosis, which is often observed in selected groups of patients with pro-coagulant imbalance.[34] Finally, the use of aspirin may reduce the risk of HCC in patients affected by chronic liver disease of unspecified etiologies, as recently reported.[35] These results are observational, with no randomization and flawed by confounding factors, but coupled with our preclinical findings, they strengthen the rationale for the design of future clinical trials to define the impact of anti-platelet therapy in chronically infected HBV patients.

CONFLICT OF INTEREST

The authors declare that they have nothing to disclose regarding funding or conflicts of interest with respect to this manuscript.

ACKNOWLEDGEMENTS

This work was partially supported by grants GR08.17 and GR08.33 from the Italian Ministry of Health.

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