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COMMENTARY

Resolving sticky relationships between platelets and lymphocytes in COVID-19: A role for checkpoint inhibitors?

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Commentary on: Paletta, et al. Platelets modulate CD4 + T-cell function in COVID-19 through a PD-L1 dependent mechanism. *Br J Haematol*. 2022;197:283-292.

Platelets have a multifaceted role in viral infections contributing to coagulopathy, thrombosis, inflammation, and immunity. Many viruses (e.g. influenza A) are able to bind to and activate platelets either through surface receptor interactions or by internalisation where Toll-like receptors recognise viral RNA.[1] In patients with coronavirus disease 2019 (COVID-19), several factors lead to platelet activation and hyperactivity. Platelets can be activated directly by the circulating inflammatory molecules (e.g. fibrinogen, interleukin [IL]-6, tumour necrosis factor alpha [TNF-α]) that are elevated during COVID-19.[2] In addition, a direct effect of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus on platelets has been described. SARS-CoV-2 virus or viral fragments can be found within platelets.[3–5] Controversy exists on how the virus enters these cells.[6]

The major receptor for SARS-CoV-2 present on epithelial cells throughout the respiratory tract is the angiotensin converting enzyme-2 (ACE-2). Using granular transcriptomic, proteomic, immunohistochemistry, and imaging methods, investigators have described the presence or the absence of the ACE-2 receptor on platelets.[3,4,7] These differences have been attributed to different ethnic populations being studied and differences in platelet isolation methods.[5,6] However, the inconsistency of ACE-2 detection on platelets suggests that alternative platelet receptors and pathways may account for these differences and explain the SARS-CoV-2 interactions with platelets. Candidate molecules and processes include CD147 (basigin) and CD26 (dipeptidyl peptidase-4) in addition to extracellular vesicles containing the virus that are released from infected endothelial cells and taken up by platelets via micro- or pinocytosis.[6] Once present within the platelet, single-stranded SARS-CoV-2 viral RNA can activate inflammatory pathways through Toll-like receptor 7.[3] Other studies have shown that platelets can internalise SARS-CoV-2 virions, directly or attached to microparticles, and this results in rapid digestion, programmed cell death, and extracellular vesicle release.[5]

Platelets from acutely ill patients with COVID-19 are highly active demonstrating increased adhesion to collagen, release of platelet granules, increasing plasma levels of platelet factor 4 and serotonin, and production of inflammatory molecules (e.g. cytokines, thromboxane).[4] Increased levels of both soluble and platelet-bound P-selectin can activate neutrophils, monocytes and lymphocytes. Similar to other infections, platelet–leucocyte aggregates develop, mediated by receptor/ligand interactions (e.g. platelet CD62P and leucocyte P-selectin glycoprotein ligand 1 [PSGL-1]) that lead to leucocyte gene expression and cellular activation.[3,8] Examples include platelet–neutrophil aggregates that induce the extrusion of neutrophil extracellular traps (NETs), which are highly thrombogenic and platelet–monocyte aggregates that induce tissue factor production by the monocytes by a P-selectin or integrin alpha IIb/beta 3 interaction enhancing the activation of the extrinsic coagulation pathway and thrombin generation.[8,9]

In this issue of the *British Journal of Haematology*, Paletta et al.[10] evaluated the immunoregulatory function of platelets on $CD4^+$ lymphocyte function in patients with COVID-19. The study cohort consisted of 62 inpatients with COVID-19 with varying severity of illness and 35 healthy donors (HD). In all, 18 patients had moderate disease and required little or no oxygen and 44 had severe disease requiring high-flow oxygen or mechanical ventilation and/or had multiple organ failure. None of the participants had been vaccinated prior to their hospitalisation. A total of 66% were treated with steroids, and 47% received anticoagulants. Blood samples were obtained an average of 8 days following the diagnosis of COVID-19 by polymerase chain reaction.

The authors first investigated the frequency of $CD4^+$ T cell–platelet aggregates in whole blood comparing HD and patients with COVID-19. The patients with COVID-19, regardless of the severity of illness, had an increased frequency of $CD4^+$ T cell–platelet aggregates that was not a function of platelet counts. To determine whether platelets have a functional effect on $CD4^+$ T-cell activity, allogeneic and autologous co-culture experiments were performed. The authors showed that while HD platelets had no effect on αCD3/αCD28-stimulated $CD4^+$ T cell TNF-α and interferon gamma (IFN-γ) secretion, co-culture with COVID-19 allogeneic platelets led to a significant decrease in T-cell CD25 expression and the production of these cytokines, while the effects on IL-17 and IL-10 production were similar (induction and inhibition respectively). Experiments with autologous platelets showed similar results. To further investigate the mechanism of inhibition, the authors surveyed existing RNAseq datasets from COVID-19 studies for inhibitory molecules and noted that programmed death-ligand 1 (PD-L1) showed the highest fold change at the gene and protein level.[3] Furthermore, platelet PD-L1 expression negatively correlated with T cell IFN-γ production. Finally, provision of a PD-L1 blocking antibody in in vitro co-culture experiments led to an increase in $CD4^+$ T cell IFN-γ production, thus restoring the function of PD-1^{high} $CD4^+$ T cells.

Although prior studies have evaluated the immune function and checkpoint molecule expression of platelets, this is the first study specifically analysing the effect of platelet PD-L1 in COVID-19.[11] Co-culture experiments described above highlight the inhibitory effect of platelets on $CD4^+$ Type 1 T-helper cell (Th1) responses and the in vitro checkpoint blockade experiments restoring $CD4^+$ T cell effector function further underscore one possible mechanism for these results. Based on the study findings, the authors suggest the use of checkpoint inhibitors (CPIs) as an adjuvant therapy in severe COVID-19.

Based on observations of peripheral lymphopenia, immune cell anergy, and apoptosis in septic patients, checkpoint molecules have been implicated in sepsis-induced immunosuppression.[12] Several preclinical and clinical studies have investigated the role of CPIs for the treatment of bacterial infections and two Phase I clinical trials have been performed testing CPIs in septic patients.[13,14] The available data to date do not support the use of CPIs in septic patients.

Additionally, several studies have suggested a role for checkpoint molecules in chronic viral infections where CPIs have been tested in clinical trials of patients with human immunodeficiency virus (HIV) and hepatitis B virus (HBV), although no approved indications exist at this time.[15,16] Conversely, studies of checkpoint molecules in murine models of acute systemic viral infections have highlighted a protective role for programmed cell death-protein 1 (PD-1), suggesting that CPIs might lead to adverse outcomes.[17] These data highlight the dilemma that while augmentation of inflammation with CPIs might provide antiviral effects, this same process might also lead to increased inflammation-mediated tissue injury.

Furthermore, recent studies have addressed the effects of prior CPI therapy on outcomes in patients with cancer who contracted COVID-19. While some studies suggest a potential deleterious effect of prior CPI therapy on COVID-19 outcomes, other studies do not support the same conclusions.[18,19] Additionally, the available literature does not always reliably address the duration of CPI therapy prior to contracting COVID-19, the type of CPI used, and whether any concomitant immune-related adverse events were present.

Finally, an increasing number of published studies have recently evaluated checkpoint molecules and their expression in blood samples from patients with COVID-19. Soluble PD-L1 levels were found to be elevated in serum samples from patients with COVID-19 compared to healthy controls and correlated with clinicopathological parameters.[20] Also, circulating T-cell subsets from patients with COVID-19 were found to be present in lower numbers and they were characterised by an inability to produce inflammatory cytokines.[21] Taken together, these data have led researchers to advocate for the therapeutic use of CPIs in patients with COVID-19.[22] Two clinical trials are currently evaluating the efficacy and safety of CPI therapy for patients with COVID-19 (National Clinical Trial number [NCT] NCT04413838, NCT04343144).

However, significant immune-related adverse events are associated with CPI therapy affecting all organ systems, including the lungs, and the occurrence of these events might not only preclude further use of these agents in infected patients but is also associated with substantial morbidity and mortality.[23] Furthermore, it is currently not known whether the risk or severity of these immune-related events is higher in patients with ongoing infection. Preclinical data suggest that provision of CPIs to animals with severe bacterial pneumonia might lead to increased pulmonary histopathological inflammation.[24]

In conclusion, Paletta et al.[10] have made novel contributions by expanding the knowledge on the role of platelet–lymphocyte aggregates and their potentially pathogenic role in COVID-19 by dampening inflammatory responses via PD-L1/PD-1 interactions. However, while studies evaluating the immunostimulatory actions of CPIs in vitro have supported a role for such immunomodulation, the effects of these interventions in patients with COVID-19 merit careful preclinical and clinical studies. Studies that have assessed the effects of prior CPI therapy on COVID-19 outcomes have not shown clear beneficial or harmful effects. The immunosuppressed phenotype of circulating immune cells might not reflect local tissue immune activity and while circulating immune cells are anergic, local effector cells might be immunocompetent. Finally, the immune response to COVID-19 is dynamic and provision of CPIs earlier or later in the course of infection might have divergent effects on outcomes.[25]

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