



Letter to the Editors-in-Chief

The homophilic CD84 receptor is upregulated on platelets in COVID-19 and sepsis

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To the editor

Platelets are the second most abundant blood cell population in circulation, derived from megakaryocytes. They are best known for their role in primary hemostasis where they sense matrix proteins and form platelet-platelet aggregates to limit blood loss at injured vessels. Platelets are increasingly recognized to mediate inflammation and immune defense, which is considered to occur by direct or indirect interactions with leukocytes. This crosstalk, also referred to as thrombo-inflammation is mediated by several platelet receptors, comprising glycoprotein (GP) VI, the main platelet receptor for collagen, CLEC-2 and Fc-receptors [1]. Recently, the homophilic immunoreceptor CD84, that belongs to the signaling lymphocyte activation molecule (SLAM)-family, got into focus with an unexpected role on platelets. Mice deficient for platelet CD84 showed no hemostatic or thrombotic phenotype in standard assays [2]. By using a murine transient middle cerebral artery occlusion (tMCAO) model, we could demonstrate that mice lacking CD84 either on platelets or on T-cells had a markedly reduced infarction area and less neurologic damage compared to wildtype animals. The soluble CD84 ectodomain, which is removed from the platelet surface by activated ADAM10 [3], increased T-cell motility *in vitro*. Moreover, increased platelet CD84 expression levels were associated with poor outcome in a cohort of stroke patients, emphasizing the role of CD84 in thrombo-inflammation [4]. Beyond this trial, the role of CD84 in other thrombo-inflammatory conditions remains enigmatic. The coronavirus disease 2019 (COVID-19) is a major contributor to the global disease burden. While acute respiratory distress is a hallmark of COVID-19, the occurrence of (micro-)thrombotic events is also frequently observed. These were shown to be triggered by the interplay of platelets with immune cells [5]. We aimed to assess the role of platelet CD84 in the thrombo-inflammatory COVID-19 disease in a prospective study, approved by the ethical committee of the University Hospital Würzburg (63/20 and 92/19) with an additional broad consent for the COVID-19 cohort. We excluded patients due to pregnancy, aplasia or ECMO therapy at any time.

We recruited a cohort of PCR-positive COVID-19 patients (male = 63 %, female = 27 %, mean age = 64a, 23 % fully vaccinated) at the ICU of the University Hospital Würzburg. Blood was withdrawn at day of

admission (t_1) and patients were followed for further four (t_2), seven (t_3), and 14 (t_4) days. In addition, we included a cohort of patients with sepsis (according to sepsis-III criteria) with comparable disease burden at t_1 . Healthy controls ($n = 13$, 27 measurements, male = 46 %, female 54 %, mean age = 27a) ≥ 18 years (no self-reported antiplatelet medication, free from acute illness) were recruited at the University Hospital Würzburg at a routinely scheduled blood withdrawal by in-house physicians. While the expression level of multiple platelet receptors (CD41/CD61, CD42a/CD42b, GPVI, CLEC-2) was comparable to healthy donors or slightly decreased (references [5,6] and data not shown), we found that CD84 expression on platelets in patients with COVID-19 or sepsis at t_1 was two-fold increased (mean GeoMFI ctrl: 206; COVID-19: 437; sepsis: 320; $p < 0.05$). Platelet CD84 surface expression levels remained elevated in COVID-19 patients during the follow-up measurements t_2 - t_4 (mean GeoMFI t_2 : 443, t_3 : 342, t_4 : 328) (Fig. 1A). In most assessed ICU patients, platelet CD84 expression peaked during their first four days at the ICU and decreased until the last individual measurement (328; $p < 0.05$), but still remained significantly elevated compared to healthy controls.

CD84 expression correlated with white blood cell count at disease onset ($r = 0.50$, $p < 0.05$), but not with platelet count or mean platelet volume (MPV), suggesting that in COVID-19 the CD84 receptor density on the platelet surface is markedly increased (Fig. 1B). In the majority of COVID-19 patients, MPV was within the reference range (indicated by the grey box in Fig. 1B) throughout the disease. Moreover, there was no correlation with inflammatory biomarkers like IL-6, procalcitonin, or fibrinogen plasma level or the 28-day mortality. CD84 upregulation cannot be readily explained by an increased mRNA upregulation, as platelet CD84 mRNA levels were decreased in a cohort of critically-ill patients with COVID-19 (log2fold change: -1.885 ; $p < 0.001$), as shown in a comprehensive study at the beginning of the pandemic [7]. Intriguingly, when we assessed total CD84 expression levels in platelet lysates derived from COVID-19 patients by immunoblotting, we found overall normal or even slightly reduced levels in a subset of $n = 7$ patients compared to $n = 4$ controls (Fig. 1C). This was confirmed by semi-quantitative densitometry of CD84 and GAPDH band intensities (Fig. 1D).

We next differentiated human mobilized CD34⁺-cells into

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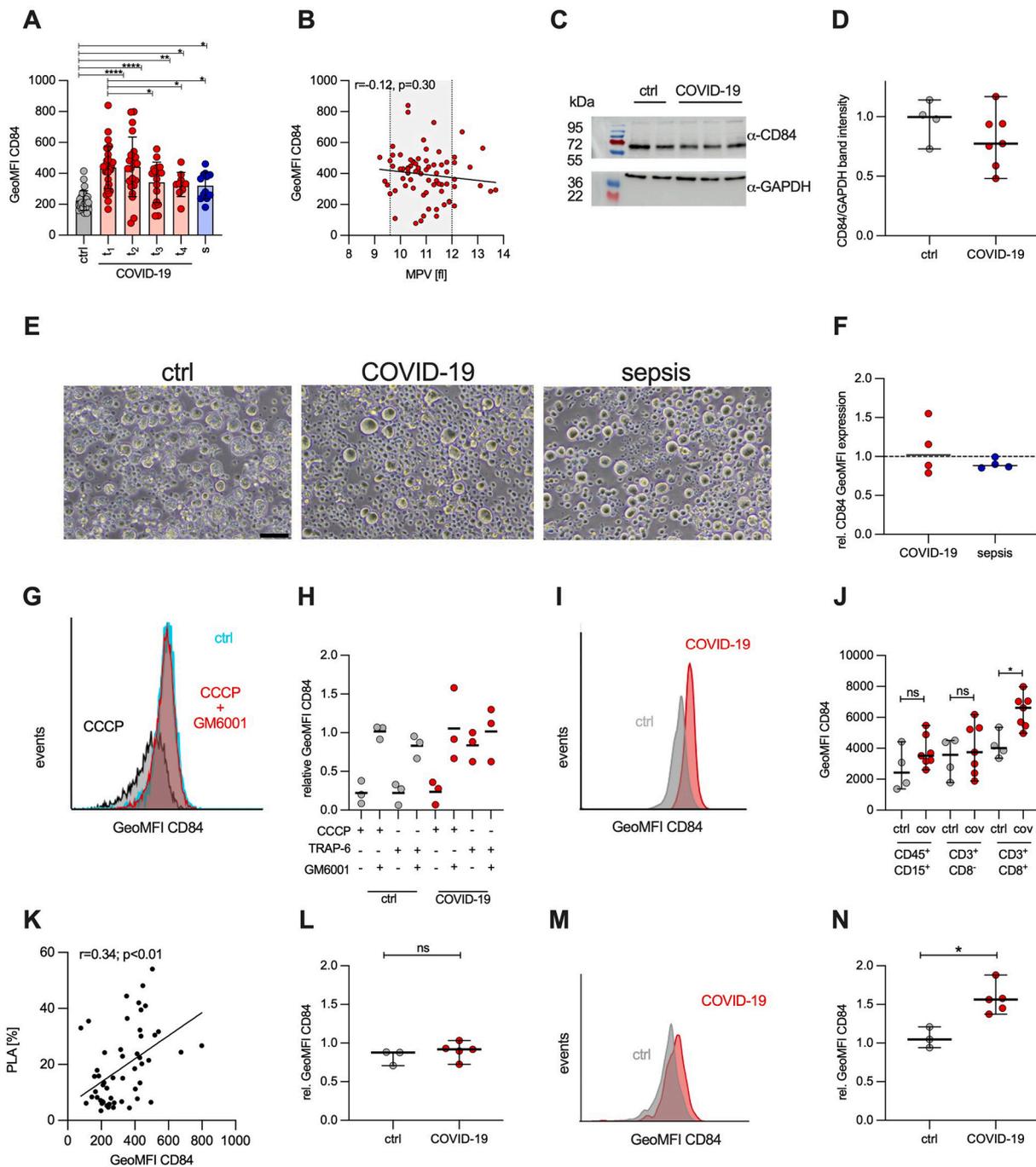


Fig. 1. (A) Platelets of patients with sepsis (s) or COVID-19 showed highly increased CD84 surface expression at 4 different time points over the disease (t_1 = day of ICU admission; t_2 = d4; t_3 = d7, t_4 = d14). (B) CD84 expression in COVID-19 patients was independent of platelet size indicated by mean platelet volume (MPV) (C–D) CD84 expression was analyzed by immunoblot in lysates of healthy controls and COVID-19 patients. CD84 signal intensity was set in relation to GAPDH. (E–F) CD84 expression on megakaryocytes (day 9) derived from human CD34⁺ cells upon co-incubation with platelet poor plasma (3 %) from sepsis patients or controls. (G–H) CD84 shedding was observed in COVID-19 patients upon CCCP [100 μ M] stimulation for 60 min at 37 °C but not upon TRAP-6 stimulation [5 μ M]. Metalloproteinase inhibitor GM6001 [100 μ M] prevented CD84 shedding. (I–J) CD84 expression on neutrophils (CD45⁺/CD15⁺) or lymphocytes (CD3⁺/CD8⁺; CD3⁺/CD8⁺) was assessed flow cytometrically. (K) Formation of platelet leukocyte-aggregates (PLA: CD41⁺/CD45⁺) and CD84 expression were assessed flow cytometrically and the values correlated by calculation of Pearson's r. (L) Platelets of healthy donors were incubated for 2 h at 37 °C in platelet-poor plasma or (M–N) whole blood of allogeneous healthy controls or COVID-19 patients prior to measuring platelet CD84 expression flow cytometrically. Representative curves are shown in panel C, E, G, I and M. Scale bars indicate 60 μ m in panel E. Data is indicated as median \pm 95 % CI. Statistical analysis was performed using (A) Kruskal-Wallis test, (E) paired *t*-test or (K, L, N) Mann-Whitney test. **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001.

megakaryocytes (MKs) in the presence of 3 % platelet poor plasma (PPP) derived from patients with either COVID-19 or sepsis, or from healthy controls. CD84 expression on culture-derived MKs was unaltered, independent of the presence of COVID-19 or sepsis-derived PPP compared to control PPP, suggesting that upregulation of CD84 in platelets is not

due to increased transcription and protein expression triggered by plasmatic factors (Fig. 1E–F).

The CD84 ectodomain can be cleaved from the platelet surface upon activation by metalloproteinases ADAM10 [3]. Dysfunctional shedding could thus explain the increased CD84 expression in patients of our

cohort. Stimulation of platelets from healthy controls with TRAP-6 or the mitochondrial-uncoupling agent CCCP [100 μ M] that activates ADAM10, resulted in reduced platelet CD84 expression. This shedding could be completely blocked by 60 min preincubation with the metalloproteinase inhibitor GM6001 [100 μ M] (Fig. 1G). We repeated this experiment with platelets from COVID-19 patients and observed a similar extent of CCCP-induced CD84 shedding as in healthy controls, but not for TRAP-6, which could in part be explained by COVID-19-based hyporesponsiveness [8] (Fig. 1H). This result suggests that dysfunctional shedding does not account for increased platelet CD84 in COVID-19, a mechanism that has been described as a cause for reduced expression of other ADAM10 substrates like CD42b or GPVI [9]. Of note, we did not detect any correlation of CD84 and CD62P surface expression on resting platelets implying that increased CD84 expression is not an epiphenomenon of platelet preactivation (data not shown).

Leukocytes express CD84 abundantly on their surface and RNA-sequencing data in mononuclear phagocytes revealed increased CD84 mRNA expression in COVID-19 samples [10]. Increased CD84 expression on leukocytes has been suggested as a positive prognostic biomarker for infect duration [11]. We therefore measured CD84 expression on CD45⁺/CD15⁺ neutrophils as well as on CD3⁺/CD8⁻ or CD3⁺/CD8⁺ lymphocytes and found upregulated CD84 on different leukocyte subsets which was most prominent on CD3⁺/CD8⁺ lymphocytes (Fig. 1I–J). These data confirm high levels of CD84 on leukocytes in COVID-19. As we found a robust correlation between platelet CD84 surface expression and leukocyte count, we hypothesized that horizontal transfer of CD84 from leukocytes to platelets could explain the upregulation of CD84 on the platelet surface. The Pearson's correlation of CD84 expression with the fraction of CD41⁺/CD45⁺ platelet leukocyte aggregates (PLA) revealed an unexpectedly strong correlation ($r = 0.34$; $p < 0.01$). This data provide another layer of evidence for our hypothesis (Fig. 1K), as an increased PLA fraction is a hallmark finding in patients with COVID-19 or sepsis.

Cellular exchange of CD84 from leukocytes to platelets could also explain the discrepant findings between our data obtained by flow cytometry in whole blood compared to the immunoblot analyses, when we lysed washed platelets, as non-covalently linked CD84 homodimers that can be detected by flow cytometry on the platelet surface could eventually dissociate during platelet isolation. We performed co-incubation experiments as previously shown [6], in which we incubated platelets of healthy donors in allogeneous PPP or whole blood of healthy controls, or derived from patients with COVID-19. To reliably identify donor-derived platelets in whole blood of recipients, donor platelets were preincubated for 30 min with a BV421-labeled CD42a antibody. We found unaltered CD84 expression in donor-derived platelets after incubation for 2 h in PPP of healthy donors or COVID-19. In contrast, CD84 levels increased by factor 1.6 after incubation in whole blood of COVID-19 patients, but not in whole blood of healthy controls, confirming cellular mediated CD84 upregulation (Fig. 1L–N).

Taken together, our findings suggest that platelet CD84 upregulation could be a meaningful biomarker in inflammatory disease conditions like COVID-19 or sepsis that occurs reliably early at disease onset. Although the sample size of our study is rather small and we fully acknowledge differences in the pathophysiology of bacterial sepsis and COVID-19, we found elevated CD84 independently of disease severity, vaccination status or underlying COVID-19 strain (Wuhan, α , δ , ϵ). Although we did not observe any correlation of therapeutic interventions with CD84 expression, ICU-therapy remains a potential confounder throughout our patient cohort. The biological relevance and the underlying mechanism for the strong upregulation remain unclear, but we hypothesize that an enforced platelet-leukocyte interplay could result in a horizontal transfer of receptor molecules between cells, which is implied by results from our co-incubation experiments. Horizontal receptor-transfer has previously been reported between non-small cell lung cancer cells and platelets exchanging PD-L1 [12]. Our data suggest that cellular crosstalk could also impact platelet receptor expression

during inflammatory disease conditions like infection. Homophilic interactions of CD84 could link immune cells to platelets and connect regulation of inflammation and immune defense with thrombus formation. Further studies are required to pave the path for deciphering the interface between leukocytes and platelets in infection.

CRedit authorship contribution statement

LJW, MD, BN and HS designed the research and analyzed data; LJW and MD, MD, BJ, AA performed experiments; KM, DW and DS provided reagents and analyzed data. LJW and HS wrote the paper.

Declaration of competing interest

BN and DS have filed a patent (PCT/EP/2020/068464) related to targeting CD84 as treatment option in ischemic diseases. All other authors do not declare any conflict of interest.

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