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SHORT COMMUNICATION
Lakerveld *et al.*, *Access Microbiology* 2023;5:000481.v3
DOI 10.1099/acmi.0.000481.v3

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Binding of respiratory syncytial virus particles to platelets does not result in their degranulation *in vitro*

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Abstract

Respiratory syncytial virus (RSV) is a major cause of severe respiratory infection in infants and the elderly. The mechanisms behind severe RSV disease are incompletely understood, but a dysregulated immune response probably plays an important role. Platelets are increasingly being recognized as immune cells and are involved in the pathology of several viruses. The release of chemokines from platelets upon activation may attract, for example, neutrophils to the site of infection, which is a hallmark of RSV pathology. In addition, since RSV infections are sometimes associated with cardiovascular events and platelets express several known RSV receptors, we investigated the effect of RSV exposure on platelet degranulation. Washed human platelets were incubated with sucrose-purified RSV particles. P-selectin and CD63 surface expression and CCL5 secretion were measured to assess platelet degranulation. We found that platelets bind and internalize RSV particles, but this does not result in degranulation. Our results suggest that platelets do not play a direct role in RSV pathology by releasing chemokines to attract inflammatory cells.

DATA SUMMARY

The data supporting the findings of this study are available within the article.

FULL-TEXT

Respiratory syncytial virus (RSV) is a major cause of severe respiratory infections, especially in infants and the elderly. Most infants experience only mild upper respiratory tract infections, but RSV infections can also develop into severe lower respiratory tract infections requiring hospitalization [1, 2]. There is no specific treatment or vaccine available for RSV, except for monoclonal antibody prophylaxis by palivizumab and its recent successor nirsevimab [3, 4]. The exact mechanisms underlying severe RSV disease are currently unknown, but a dysregulated immune reaction probably contributes to lung damage (reviewed in [5]). Excessive infiltration of immune cells into the lungs is often seen in severe RSV disease [6, 7]. In order to develop novel treatment options and vaccines, a better understanding of RSV immunopathology is necessary.

Various types of immune cells may be involved in immunopathology. Although platelets are primarily known for their function in thrombosis and haemostasis, they have recently received more attention for their immunological role. A broad range of pattern-recognition receptors (PRRs) are expressed on platelets, including toll-like receptors (TLRs), C-type lectin receptors (CLRs) and NOD-like receptors (reviewed in [8]). Upon activation, platelets release the contents of α-granules and dense granules containing immunomodulatory cytokines, chemokines and growth factors. These molecules can modulate the immune response indirectly by attracting immune cells to the site of infection, thereby contributing to cellular infiltration. In addition, when platelets become activated, P-selectin, normally residing on the membrane of cytosolic α-granules, becomes exposed on the platelet surface. Platelets can directly interact with other immune cells via binding of P-selectin to its receptors present on neutrophils, monocytes and macrophages (reviewed in [8]).

Received 09 August 2022; Accepted 30 June 2023; Published 13 July 2023
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Keywords: P-selectin expression; platelets; RSV; respiratory syncytial virus.
Abbreviations: CCL2/CCL5, C-C motif chemokine ligand 2 or 5; CLR, C-type lectin receptor; CX3CR1, CX3C chemokine receptor 1; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; GFP, green fluorescent protein; HIV, human immunodeficiency virus; PRP, platelet-rich plasma; RSV, respiratory syncytial virus; TLR, toll-like receptor; TRAP-6, thrombin receptor activating peptide-6; VEGF, vascular endothelial growth factor.

The immunomodulatory role of platelets has already been shown for several viral infections, such as dengue virus, human immunodeficiency virus (HIV) and influenza virus [9–11]. Platelet hyperactivity has also been implicated in the severe pathology of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infections [12, 13]. There are several indications suggesting that platelets may also play a role in RSV infections. Interestingly, increased expression of genes associated with platelets was found in RSV lower respiratory tract infections in infants [14]. Cardiovascular complications, to which platelets may contribute, have also been associated with RSV infections (reviewed in [15]). Furthermore, multiple receptors known to bind RSV are expressed on the platelet membrane, such as TLR4, CX3CR1 and DC-SIGN [16–18]. Additionally, platelet CLRs potentially bind glycans present on the highly glycosylated RSV surface proteins [19]. While platelets are normally not present in the healthy lung lumen, RSV infection probably leads to epithelial damage enabling various immune cells, including platelets, to enter the alveolar space where they will encounter RSV particles. Likewise, platelets have been found in the lungs of allergen-challenged mice and in mice with LPS-induced inflammation [20, 21]. Furthermore, lungs have been found to harbour platelet precursor cells (megakaryocytes), suggesting that some platelet production occurs in this tissue in addition to the bone marrow as a main production site [22]. In this study, we examined whether platelets may be involved in RSV disease, specifically whether exposure to RSV particles leads to platelet degranulation *in vitro*.

Washed human platelets were isolated from 3.2% sodium citrate anticoagulated whole blood from healthy adult volunteers as described previously [23]. People using aspirin within 2 weeks prior to blood drawing were excluded. Age and sex were not disclosed and deemed irrelevant for this study, although it is noted that a difference in platelet activation between boys and girls during RSV-induced bronchiolitis has been described previously [24]. Briefly, whole blood was centrifuged at 160 ***g*** for 15 min without a brake to obtain platelet-rich plasma (PRP). PRP was further centrifuged in the presence of anticoagulants to ultimately obtain washed platelets, which were resuspended in HEPES Tyrode buffer at a concentration of 100×10^6 platelets ml^{-1}. Platelets were rested for 30 min before continuing with experiments.

Human RSV-A2 (ATCC VR-1540), RSV-98-25147 X (RSV-A strain from the Netherlands, 1998, here referred to as RSV-X; GenBank FJ944820.1) and recombinant RSV-X-GFP [25] were propagated in HEp-2 cells (ATCC Cat. no. CCL-23, RRID:CVCL_1906). Virus stocks were purified between layers of 10 and 50% sucrose by ultracentrifugation and subsequently snap-frozen. Virus stored at −80 °C was rapidly thawed at 37 °C shortly before use. The 50% tissue culture infective dose ($TCID_{50}$) per millilitre was determined on Vero cells (ATCC Cat. no. CCL-81, RRID:CVCL_0059) using the Spearman and Karber method [26] and converted to plaque-forming units (PFU) per millilitre by multiplying by 0.69.

To assess RSV binding, washed platelets were incubated for 1 h at 37 °C with sucrose-purified RSV-A2 diluted in HEPES Tyrode buffer in different concentrations, indicated as plaque-forming units determined on Vero cells (PFU_{Vero}) per platelet. As a mock control, only HEPES Tyrode buffer was added to the platelets. Following incubation, platelets were stained with an FITC-labelled antibody specific for the RSV attachment protein G (Merck Millipore Cat. no. MAB858-2F-5, clone 131-2G) and cell surface markers anti-human CD41-PE (BioLegend Cat. no. 303706, RRID:AB_314376, clone HIP8) and anti-human CD45-BUV395 (BD Biosciences Cat. no. 563792, RRID:AB_2869519, clone HI30) and analysed by flow cytometry on an LSR Fortessa X-20 (BD Biosciences). Flow cytometry plots detailing the gating strategy for single CD41+CD45− platelets can be found in Fig. 1. Increasing amounts of RSV resulted in an increasing percentage of FITC-positive platelets up to approximately 15% (n=6), thus indicating platelets with surface-attached RSV particles (Fig. 2a). To investigate whether platelets were able to bind different RSV strains, both RSV-A2 and RSV-X were added to platelets at a ratio of 0.1 PFU_{Vero} per platelet for 1 h (n=4). Fig. 2(b) shows that platelets can bind both RSV-A2 and RSV-X.

Platelets are known to exhibit endocytic activity [11, 27]. To determine whether platelets can also internalize RSV particles, platelets were fixed and permeabilized with a cytofix/cytoperm kit (BD Biosciences) following 1 h of incubation and stained for RSV as described above (n=4). Fig. 2(c) shows a clear increase in the percentage of platelets positive for RSV when permeabilized compared to the non-permeabilized controls (18 and 10%, respectively). This indicates that besides binding of RSV to the platelet surface, RSV particles are also localized inside the platelet and are thus internalized.

P-selectin is a well-known platelet activation marker which resides on the membrane of α-granules in resting platelets. After activation and granule release, P-selectin is exposed on the surface of platelets, where it can be measured with flow cytometry using anti-human CD62P-PE/Cy7 (BioLegend Cat. no. 304922, RRID:AB_2572028, clone AK4) to obtain an indication of the extent of platelet degranulation. When exposing platelets to increasing amounts of RSV, only a small fraction of platelets (1–3%, n=6) show surface expression of P-selectin (Fig. 2d). Furthermore, the minor increase that is observed at the highest RSV concentrations appears to be the result of sucrose present in the virus stocks, as it is also observed in the sucrose control condition ('Suc' in Fig. 2d) where a similar concentration of sucrose was added as was present at the highest RSV concentration. Using thrombin receptor activating peptide-6 SFLLRN (TRAP-6, BACHEM Cat. no. H-8365) at a concentration of 25 µM as a positive control, we show that the platelets used in our experiments are indeed able to detectably express P-selectin on their surface, as approximately 70% of platelets stain positive for P-selectin after TRAP-6 incubation. Surface expression of CD63, present on the membrane of dense granules in resting platelets, is another well-known platelet degranulation marker. Similar to P-selectin, we found that CD63 [stained with anti-human CD63-PerCP/Cy5.5 (BioLegend Cat. no. 353020, RRID:AB_2561685, clone H5C6)]

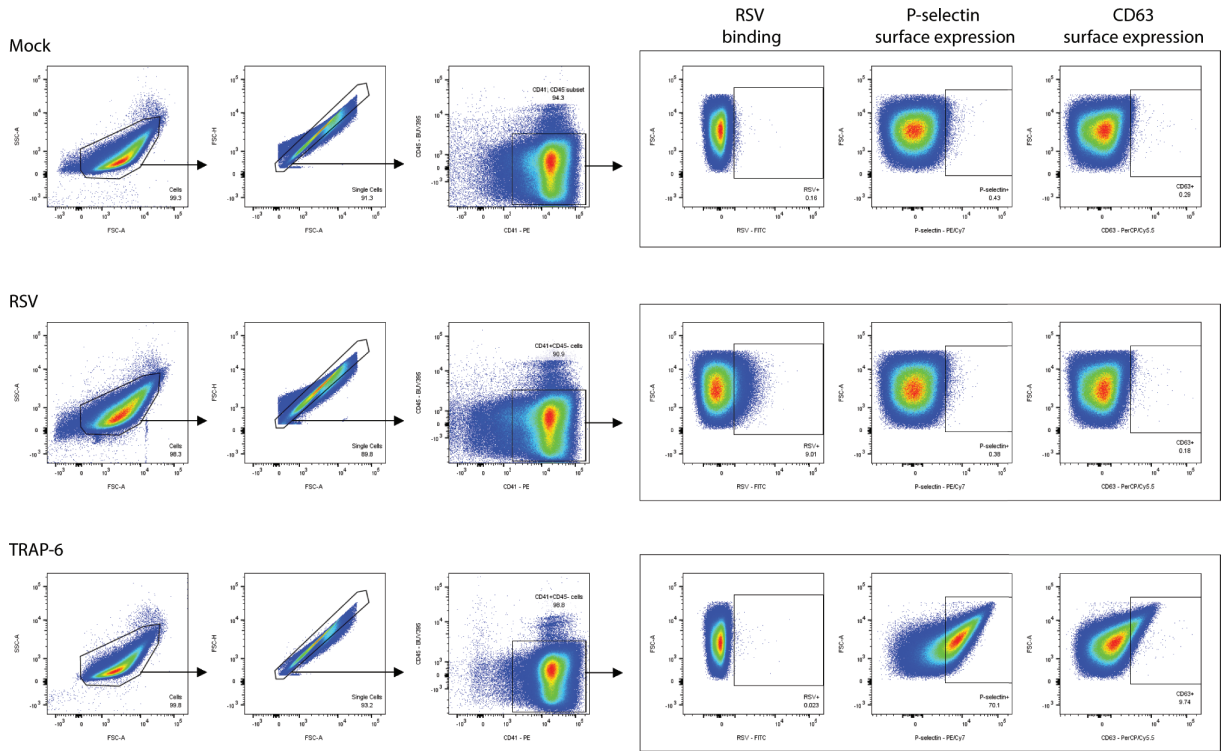

Fig. 1. Gating strategy to assess RSV binding (FITC-conjugated 131-2G) and P-selectin (CD62P, PE/Cy7-conjugated AK4) and CD63 (PerCP/Cy5.5-conjugated H5C6) surface expression on single CD41+ (PE-conjugated HIP8) CD45– (BUV395-conjugated HI30) platelets after mock, RSV or TRAP-6 incubation. Flow cytometry plots are shown for one representative donor and made using FlowJo software (RRID:SCR_008520).

is not expressed on RSV-exposed platelets (n=5) while it is expressed on TRAP-6-stimulated platelets (Fig. 2e). Together, these results suggest that RSV particles do not induce degranulation of platelets.

Platelet degranulation results in the release of various molecules including cytokines, chemokines and growth factors. As an example, Fig. 2(f) shows that secretion of the platelet-associated chemokine CCL5 (determined using a custom LegendPlex assay; Biolegend) is not increased following a 1 h incubation of platelets with RSV particles, while CCL5 secretion does show an increase upon TRAP-6 stimulation (n=6). This finding is in line with the absence of an effect of RSV incubation on P-selectin and CD63 surface expression and supports the notion that RSV exposure does not result in platelet degranulation. Strikingly, we did observe increased concentrations of several other (platelet-related) growth factors and chemokines (e.g. VEGF and CCL2) in the platelet supernatant following incubation with RSV. However, upon further inspection, these molecules turned out to originate from the virus stocks and were thus in fact not platelet-derived. Interestingly, others have previously also shown co-purification of host proteins with RSV, speculating that these were either virion-associated or carried within extracellular vesicles [28, 29]. These findings highlight the importance of including proper controls when working with cell-culture-derived materials such as virus stocks.

Since we observed internalization of RSV particles by platelets, we also examined whether RSV was able to replicate in platelets, as has been shown before for dengue virus [30]. To this end, we added (untreated or UV-inactivated) recombinant RSV encoding green fluorescent protein (GFP) to platelets (n=2). The GFP signal becomes visible when transcription and translation of viral mRNA occurs (which should no longer occur following UV inactivation) and is thereby indicative of the initiation of the viral replication cycle. Up to 2 days after the addition of RSV, the percentage of GFP-positive platelets (as measured with flow cytometry) increased to only 1.5% in the untreated RSV condition while the UV-inactivated RSV condition had already reached approximately 1.0% (Fig. 3). This finding suggests strongly that most of the observed GFP signal was due to background, for example uptake of GFP-containing material from the virus stock. RSV replication in platelets therefore appears to be extremely limited and – even if it were to result in infectious progeny – is probably biologically irrelevant.

With this study, we show that platelets are able to bind and internalize RSV particles, but do not release their granules after exposure to RSV *in vitro* and consequently do not appear to secrete chemokines to attract immune cells to the site of infection. Furthermore, very limited replication of internalized RSV could be observed in platelets *in vitro*, which is probably biologically irrelevant. In summary,

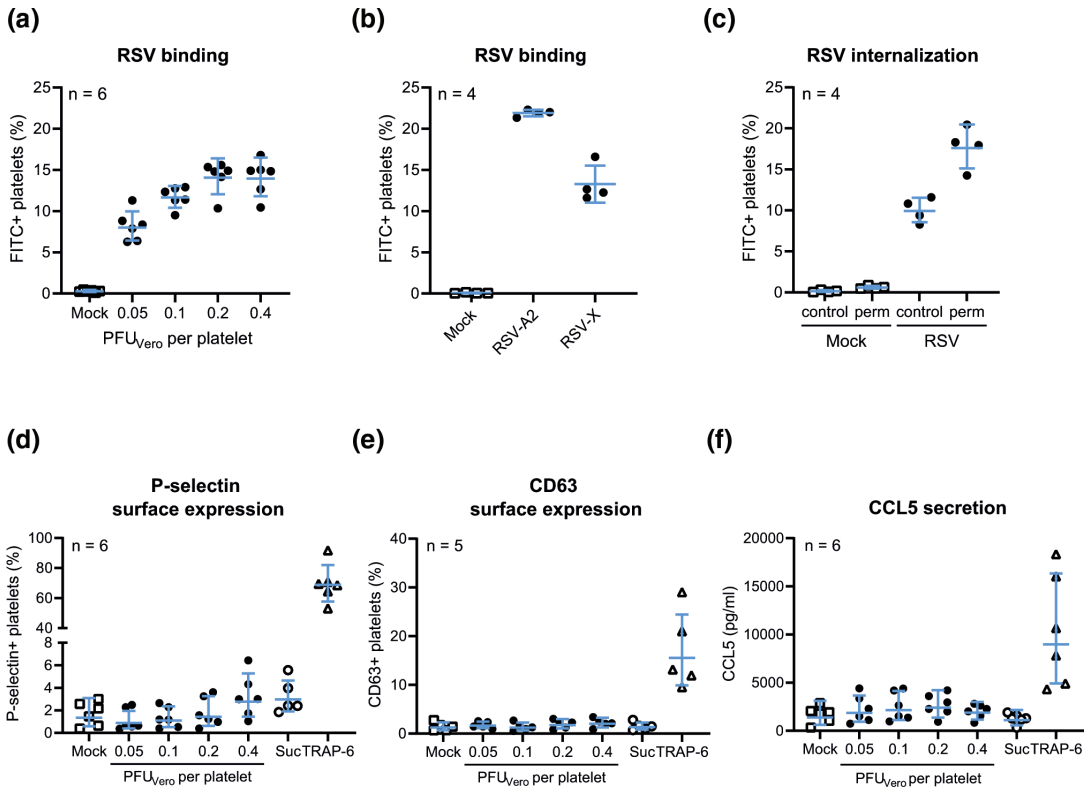

Fig. 2. Platelets bind and internalize RSV particles, which does not lead to platelet degranulation *in vitro*. (a) Washed platelets were incubated for 1 h with sucrose-purified RSV-A2 at the indicated concentrations and analysed by flow cytometry after staining with an FITC-conjugated antibody specific for the RSV attachment protein G (131-2G). Graphs show the percentage of FITC-positive platelets of different donors (*n*=4–6). (b) Washed platelets were incubated for 1 h with 0.1 PFU_{Vero} per platelet of RSV strains A2 (ATCC VR-1540) or X (GenBank FJ944820.1) and analysed as in (a). (c) Following 1 h of incubation with RSV-A2 at 0.1 PFU_{Vero} per platelet, platelets were fixed and permeabilized with a cytofix/cytoperm kit, stained as in (a) and measured with flow cytometry to assess RSV internalization. Following 1 h of incubation with RSV-A2 at 0.1 PFU_{Vero} per platelet, platelet degranulation was assessed by measuring P-selectin (d) and CD63 (e) surface expression and CCL5 secretion (f). Each data point indicates the average of technical duplicates from a single platelet donor. Abbreviations: perm, permeabilized; PFU, plaque-forming units; suc, sucrose control; TRAP-6, thrombin receptor activating peptide-6.

the observed lack of degranulation and very limited viral replication suggests that a harmful role for platelets in RSV pathology is improbable. In contrast, it is conceivable that RSV in fact suppresses platelet activation, but further experiments are necessary.

The mechanism behind the interaction between RSV and platelets remains unclear. As mentioned previously, platelets contain several receptors known to be able to bind RSV (TLR4, CX3CR1 or DC-SIGN) [16–18]. RSV might bind to platelets through these receptors, but a less specific interaction in which the glycans of RSV surface proteins bind to CLRs on platelets is also possible. Additionally, the RSV attachment protein contains a heparin binding domain that can bind to heparan sulphate proteoglycans [31, 32]. This is also a potential mode of interaction, as has been shown for dengue virus binding to platelets [30].

In contrast to the study of Kullaya *et al.,* in which RSV was shown to induce P-selectin expression on platelets [33], we did not find an induction of P-selectin surface expression after RSV exposure. This discrepancy may be explained by the fact that we exposed the platelets to a much lower concentration of RSV particles, which we believe is physiologically more relevant. With these RSV concentrations, we were able to observe a plateau in RSV-positive platelets, while activity markers remained entirely unaffected. This observation suggests strongly that platelet degranulation is not induced by RSV. Furthermore, we show that sucrose has a small influence on P-selectin expression, which might have played a role in the study of Kullaya *et al.* that also used sucrose-purified RSV. Although all our experiments were performed *in vitro*, which does not completely mimic the *in vivo* situation, the positive control TRAP-6 showed that activation was indeed possible under these *in vitro* conditions.

Whereas platelets may not play a detrimental role in RSV disease severity by attracting immune cells, they might instead exert a protective role by binding and internalizing RSV particles. It has previously been shown that platelets are able to engulf and eliminate pathogens, contributing to pathogen clearance [11, 27, 34]. Indeed, Kullaya *et al.* showed that in the presence of platelets, a lower number of monocytes become infected with RSV, resulting in reduced monocyte activation [33]. Additionally, platelets can also present

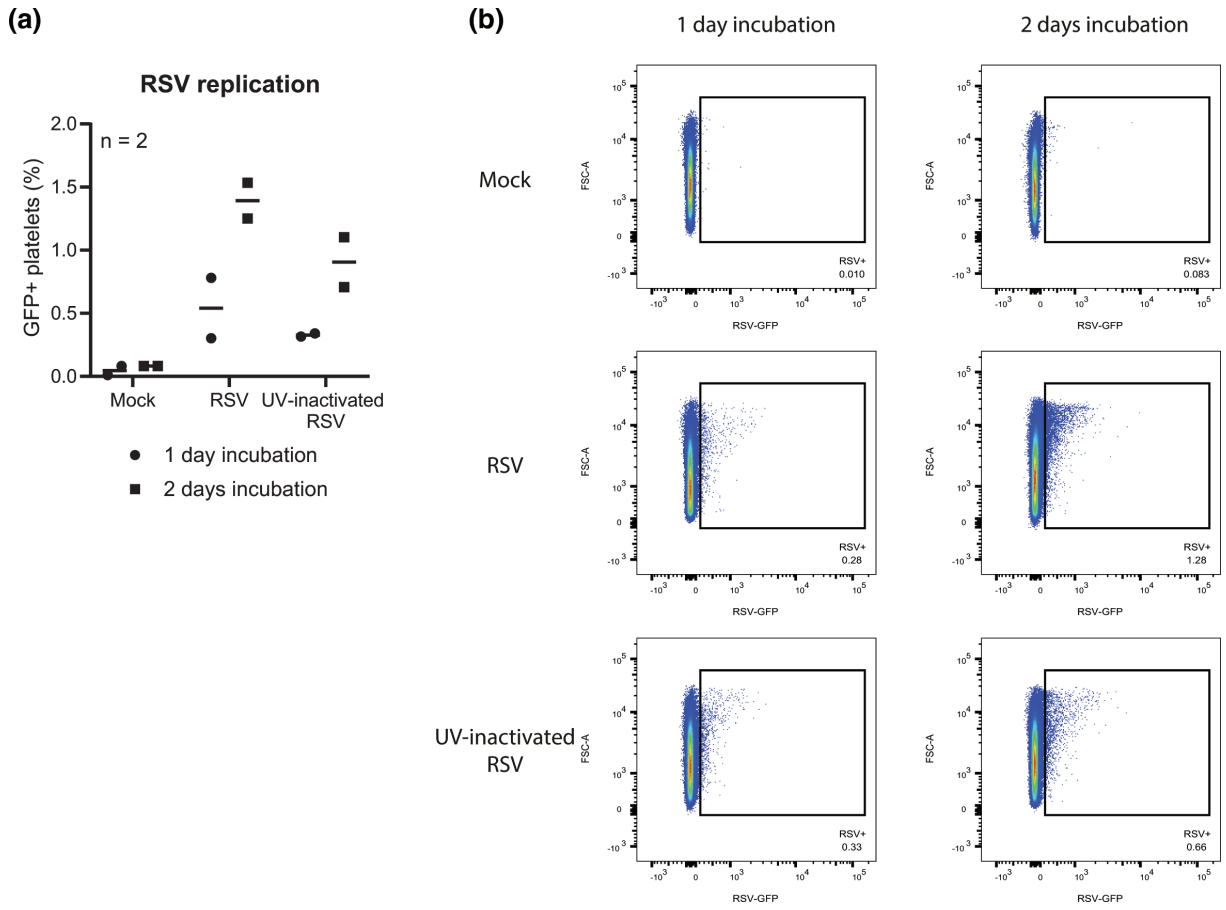

Fig. 3. RSV replication in platelets is limited. CD41+CD45− platelets were gated as shown in Fig. 1. Untreated or UV-inactivated RSV-GFP was added to washed platelets from healthy donors (n=2) at a concentration of 0.1 PFU_{Vero} per platelet and incubated for up to 2 days. (a) Graph showing the percentage of GFP-positive platelets, potentially indicating expression of virus-encoded genes and thus the initiation of viral replication. Each data point indicates the average of technical duplicates from a single platelet donor. (b) Flow cytometry plots for one representative donor made using FlowJo software.

pathogens to phagocytes and dendritic cells and limit their dissemination into the bloodstream [35, 36]. As RSV is mainly a respiratory pathogen, platelets may present RSV to alveolar macrophages. However, considering the absence of RSV-induced P-selectin surface expression, platelet binding to other immune cells may be limited.

Viral infections are sometimes associated with thrombocytopenia, a decrease in platelet counts [27, 37]. During RSV infection, generally no differences in platelet counts have been noted [38], although sporadically thrombocytosis is observed, an increase in platelet counts [39]. Therefore, binding of RSV particles to platelets does not appear to result in significant changes to platelet counts *in vivo*. Instead of functioning to limit infection throughout the body, some viruses (e.g. HIV) actually use platelets as a vehicle to disseminate through the entire body and promote viral spread [40]. It is highly unlikely that platelets play such a role to any significant extent during RSV infection, as RSV RNA has only rarely been found outside the respiratory tract [41, 42].

In conclusion, based on our findings we consider it unlikely that platelets play a direct role in RSV pathology, especially not a detrimental one. The fact that platelets are able to bind RSV particles might limit viral spread, but at the assessed concentrations platelet degranulation is not induced by RSV and these cells are therefore unlikely to contribute significantly to the excessive cellular infiltration observed during severe RSV disease.

Funding information
This work was funded by the Dutch Ministry of Health, Welfare, and Sports (VWS).

Acknowledgements
We kindly thank the blood donors for contributing to this study, and Rick Kapur, Teun Guichelaar and Jørgen de Jonge for helpful discussions.

Author contributions
A.J.L.: conceptualization, investigation, formal analysis, writing – original draft, writing – review & editing, visualization; E.A.v.E.: conceptualization, formal analysis, writing – review & editing, supervision; P.B.v.K.: conceptualization, formal analysis, writing – review & editing, supervision, funding acquisition.

Conflicts of interest
The authors declare no conflicts of interest.

Ethical statement
This study was conducted according to the principles described in the Declaration of Helsinki, and for the collection of samples and subsequent analyses, all blood donors provided written informed consent. Blood samples were processed anonymously and the research goal, primary cell isolation, required no review by an accredited Medical Research Ethics Committee (MREC), as determined by the Dutch Central Committee on Research involving human subjects (CCMO).

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Peer review history

VERSION 2

Editor recommendation and comments

https://doi.org/10.1099/acmi.0.000481.v2.3

Adam P Roberts; Liverpool School of Tropical Medicine, UNITED KINGDOM

Date report received: 30 June 2023
Recommendation: Accept

Comments: The manuscript is improved following the author's revision and is now acceptable for publication in Access Microbiology

SciScore report

https://doi.org/10.1099/acmi.0.000481.v2.1

iThenticate report

https://doi.org/10.1099/acmi.0.000481.v2.2

Author response to reviewers to Version 1

Response to reviewer comments

Binding of respiratory syncytial virus particles to platelets does not result

in their degranulation in vitro - Anke J. Lakerveld, Elisabeth A. van Erp, Puck B. van Kasteren

We thank the editor and reviewers for their careful examination of our work and the helpful suggestions. We appreciate their acknowledgement of the importance of our research question and recognizing the validity of our findings. We have addressed all questions and comments below (in blue).

Reviewer 1 Comments to Author: This study by Lakerveld et al., reports experiments investigating the interaction with purified RSV (an important human pathogen) particles with freshly isolated human platelets as it has emerged recently that platelets play important roles in host response to - and control of - viral infections. This is a focused and convincing set of experiments, that use "gold-standard" approaches to address this important question alongside critical control experiments. The authors convincingly show evidence that RSV fails to classically activate platelets, despite binding and entering into them. I have a few suggestions that may help clarify areas in this manuscript prior to publication. My comments largely come from an interest in this area of research stemming from such an engaging article.

Comments:

Can the authors provide images of platelets during each of the treatments?

While this is an interesting suggestion, we have not made images of the platelets during any of these experiments and therefore we are currently unable to provide these.

The authors cannot comment on whether RSV replicate inside platelets using their current approach. While yes this system should only express GFP during replication, GFP detection by flow could be explained uptake of GFP-containing material during incubation. Yes this level of infection is much lower than would occur in highly permissive cell lines, it would be of great interest if RSV replicated at even low levels, therefore, the authors could:

Use UV inactivated virus

Bind with RSV and wash off and incubate and measure GFP

The reviewer makes an excellent point that the observed GFP signal might be explained by the uptake of GFP-containing material. In fact, we had actually already included a UV-inactivated control in our experiment but did not include this control in the original manuscript because we believed the observed GFP positivity in the untreated condition was already too low in itself to represent biologically relevant replication. However, we completely agree with the reviewer that with the inclusion of these additional data (line 132-142, figure 3) we can make an even stronger argument that replication of RSV in platelets is indeed extremely limited.

Methods: did the authors use fresh pure particles or did they freeze/thaw before challenge?

We acknowledge that this is relevant information for the reader and added the following information to the manuscript (line 77-78): The sucrose-purified virus is snap-frozen quickly after purification. Subsequently, the viruses are stored at -80°C until further use. Shortly before use, the virus is thawed rapidly at 37°C, which is a common practice in the RSV field.

This reviewer disagrees on the MOI interpretation especially when comparing between virus stocks. As this work is largely independent of infectivity (maybe), a greater emphasis should be placed on particles/genome copies rather than MOI/pfu or tcid50. Can the authors comment on this, and modify any MOI- based conclusions?

We agree with this reviewer that MOI/pfu cannot be used to directly compare between virus stocks in this context. To avoid any suggestion of quantitatively comparing between virus stocks, we removed the sentence mentioning that the percentages of positive platelets differ between different virus strains (line 93-94).

Are these platelets from a single donor? Especially thinking about the infection experiment.

We thank the reviewer for pointing out that the number of individual donors was not immediately clear from the manuscript text and have made several adaptations to address this. The experiments of figure 2 are performed with platelets of 4 to 6 donors (as indicated in the figure). The infection experiment (figure 3) was performed with platelets of 2 donors (as indicated in the figure). The number of donors was also mentioned in the figure legend of figure 2. We now added this information to the figure legend of figure 3 (line 344). For additional clarity, we added the number of donors to the manuscript main text as well (lines 90, 93, 97, 105, 116, 123, 135).

Can the authors provide a few lines on the passage history of their X strain, as it is not one that I had come across before and it is hard to find out information online. In particular, how many passages on what cells before cloning to cDNA? And then, how many passages and what cells before stocks used here?

The recombinant virus has been passaged approximately 5 times on Vero cells before cloning. After cloning, 6 passages on Vero cells were performed. As indicated, our virus stocks were ultimately produced on Hep2 cells. Although some cell culture adaptation has likely occurred, we have shown previously that, similar to RSV-A2 and an RSV-B strain, RSV-X grows well on primary differentiated airway epithelial cells, a model resembling the human airways, and induces similar cytokine responses (Yu et al., mSphere 2020, DOI: 10.1128/mSphere.00577-20).

The authors should be cautious on their conclusions on the role of platelets in RSV pathology, as they only looked at a few inflammatory mediators, and more interestingly, this work raises the possibility that RSV actively suppresses platelet activation. Could this be looked at by co-incubation of RSV with positive control?

This is certainly an interesting suggestion. We have added a sentence considering this possibility (line 148-149). We have not performed experiments with co-incubations, but whether RSV actively suppresses platelet activation is definitely of interest to investigate in the future.

It is interesting the detection of RSV associated chemokines. Can the authors speculate the reasons? Are they associated with virions as suggested, or could they come from additional non-viral structures like extracellular vesicles? Is there a chance that this indicates the sucrose purification did not work appropriately?

We believe that extracellular vesicles are indeed, as this reviewer suggests, a likely source of the chemokines in our sucrose-purified virus stocks. These vesicles have similar biophysical properties as virus particles (size, lipid bilayer), and will therefore likely be collected together with the virus particles during purification using a density gradient. After the discovery of chemokines in the used virus stock, we also tested other sucrose-purified RSV stocks, in which we found similar results. Additionally, Radhakrishnan et al. previously found at least 25 host proteins to be co-purified with RSV during sucrose-purification. These were mainly proteins associated with energy pathways, the cytoskeleton, and heat-shock proteins (Radhakrishnan et al., Mol Cell Proteomics 2010, DOI: 10.1074/mcp.M110.001651). Furthermore, it has also previously been described in literature that cytokines might sometimes still be present after sucrose purification (Ajamian et al., J Leukoc Biol. 2020, DOI: doi: 10.1002/JLB.4MA0320-621R). Ajamian et al. found that CCL5 persisted in their purified stocks, whereas TNF and IFN-a did not. We have added a sentence referring to these papers in the manuscript text. Co-purification of host proteins therefore seems to be

an inherent feature of sucrose-purification - even when appropriately performed - which is to our knowledge not generally recognized in the field.

Reviewer 3 Comments to Author: Comments on Lakerveld et al

This manuscript focusses on respiratory syncytial virus (RSV), A clinical challenge public health risk without significant treatment. The authors investigate a very well justified aspect of the immune response to RSV and show, convincingly, that platelets are unlikely to play a role in RSV infection.

Minor comments for the authors to address;

Line 67; Please include a brief summary of the possibility that the gender of the platelet donors may affect the outcome of the study, however unlikely this may be and provide a suitable reference to lead the reader to the data on gender effects of platelets; Gender differences in platelets from girls and boys with bronchiolitis were found by Tarissi De Jacobis et al., in a pilot study. (De Jacobis et al., Ital J Pediatr. 2020 Mar 6;46(1):29. doi: 10.1186/s13052-020-0792-x.PMID: 32143677).

We added a sentence about this possibility (line 68-69) and added reviewer's suggested reference.

VERSION 1

Editor recommendation and comments

https://doi.org/10.1099/acmi.0.000481.v1.5

Adam P Roberts; Liverpool School of Tropical Medicine, UNITED KINGDOM

Date report received: 27 April 2023
Recommendation: Minor Amendment

Comments: The work presented is clear and the arguments well formed. This study would be a valuable contribution to the existing literature. This is a study that would be of interest to the field and community. The reviewers have highlighted minor concerns with the work presented. Please ensure that you address their comments.

Reviewer 2 recommendation and comments

https://doi.org/10.1099/acmi.0.000481.v1.4

Adam Roberts; Liverpool School of Tropical Medicine, UNITED KINGDOM
https://orcid.org/0000-0002-0760-3088

Date report received: 27 April 2023
Recommendation: Minor Amendment

Comments: Comments on Lakerveld et al This manuscript focusses on respiratory syncytial virus (RSV), A clinical challenge public health risk without significant treatment. The authors investigate a very well justified aspect of the immune response to RSV and show, convincingly, that platelets are unlikely to play a role in RSV infection. Minor comments for the authors to address; Line 67; Please include a brief summary of the possibility that the gender of the platelet donors may affect the outcome of the study, however unlikely this may be and provide a suitable reference to lead the reader to the data on gender effects of platelets.

Please rate the manuscript for methodological rigour
Very good

Please rate the quality of the presentation and structure of the manuscript
Very good

To what extent are the conclusions supported by the data?
Strongly support

Do you have any concerns of possible image manipulation, plagiarism or any other unethical practices?
No

Is there a potential financial or other conflict of interest between yourself and the author(s)?
No

If this manuscript involves human and/or animal work, have the subjects been treated in an ethical manner and the authors complied with the appropriate guidelines?
Yes

Reviewer 1 recommendation and comments

https://doi.org/10.1099/acmi.0.000481.v1.3

Connor Bamford; Queen's University Belfast, Wellcome-Wolfson Institute for Experimental Medicine, UNITED KINGDOM

Date report received: 29 October 2022
Recommendation: Minor Amendment

Comments: This study by Lakerveld et al., reports experiments investigating the interaction with purified RSV (an important human pathogen) particles with freshly isolated human platelets as it has emerged recently that platelets play important roles in host response to - and control of - viral infections. This is a focused and convincing set of experiments, that use "gold-standard" approaches to address this important question alongside critical control experiments. The authors convincingly show evidence that RSV fails to classically activate platelets, despite binding and entering into them. I have a few suggestions that may help clarify areas in this manuscript prior to publication. My comments largely come from an interest in this area of research stemming from such an engaging article. Comments: Can the authors provide images of platelets during each of the treatments? The authors cannot comment on whether RSV replicate inside platelets using their current approach. While yes this system should only express GFP during replication, GFP detection by flow could be explained uptake of GFP-containing material during incubation. Yes this level of infection is much lower than would occur in highly permissive cell lines, it would be of great interest if RSV replicated at even low levels, therefore, the authors could: Use UV inactivated virus

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Please rate the manuscript for methodological rigour
Good

Please rate the quality of the presentation and structure of the manuscript
Very good

To what extent are the conclusions supported by the data?
Partially support

Do you have any concerns of possible image manipulation, plagiarism or any other unethical practices?
No

Is there a potential financial or other conflict of interest between yourself and the author(s)?
No

If this manuscript involves human and/or animal work, have the subjects been treated in an ethical manner and the authors complied with the appropriate guidelines?
Yes

SciScore report

https://doi.org/10.1099/acmi.0.000481.v1.1

iThenticate report

https://doi.org/10.1099/acmi.0.000481.v1.2