Megakaryocyte infection by SARS-CoV-2 drives the formation of pathogenic afucosylated IgG antibodies in mice

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Abstract

Despite more than 90% of total plasma fucosylated IgG, specific IgGs with low core fucosylation are found sporadically in response to enveloped virus infections and to alloantigens on blood cells. IgG responses with low core fucosylation are directly pathogenic in SARS-CoV-2 and dengue infections. In COVID-19, formation of IgG with low core fucosylation (afucosylated IgG) against spike protein (S) predicts and directly mediates disease progression to severe form. Low fucosylation of IgG causes increased antibody-dependent cellular toxicity mediated by intense FcγR-mediated stimulation of platelets, monocytes, macrophages, and natural killer cells. The mechanism of afucosylated IgG formation has remained elusive thus far in COVID-19, dengue infection, and other disorders. This study demonstrates that infection of megakaryocytes by SARS-CoV-2 drives the formation of pathogenic anti-S afucosylated IgGs, causing pulmonary vascular thrombosis, acute lung injury, and death in Fcγ-expressing mice.

Keywords: COVID-19, SARS-CoV-2, Antibody, Afucosylated IgG, Megakaryocytes.

Introduction

The coronavirus SARS-CoV-2 is the causative agent of the global coronavirus 2019 (COVID-19) pandemic which has caused a severe threat to public health since its inception. SARS-CoV-2 infection causes a mild acute disease in the majority of those infected, but progresses to severe and fatal disease in a subset [1]. Extensive work has been done to elucidate the precise events responsible for disease progression in COVID-19. Two specific early events that accurately predict disease progression have been independently identified. These events include (1) the early interaction between SARS-CoV-2 and megakaryocytes (MKs) in the bone marrow [2,3], and (2) the early production of a distinct form of non-neutralizing antibody against SARS-CoV-2 spike protein that drives the immunopathology of progressive disease [4–6].

Multiple studies have shown that the quality of antibodies produced early on in response to SARS-CoV-2 spike protein differs between individuals, and the production of a distinct form of anti-spike IgG early in the illness accurately predicts disease progression [4–6]. Specifically, the glycosylation pattern of the Fc component of IgGs formed against the spike protein was shown to determine the fate of outpatients studied in two prospective cohorts [4,6–8]. Individuals who, due to unclear factors, produced IgGs with low core fucosylation (afucosyalted IgGs) against the spike protein early in the illness as outpatients consistently progressed to hospitalization and severe COVID-19 [4,6,8]. Those who instead formed anti-spike IgGs with normal levels of core fucosylation mounted an appropriate immune response to SARS-CoV-2 early on, experienced only a mild course and did not progress to severe COVID-19 [4–6].

Mechanistically, low core fucosylation of anti-spike IgGs in COVID-19 is known to trigger a potent pro-inflammatory cascade of cytokine production and lung immunopathology driven by intense FcγR-mediated stimulation of macrophages, monocytes, natural killer cells, and platelets [5,6]. Intriguingly, in individuals who produced afucosylated IgG against the spike protein (who later on progressed to severe disease), the low core fucosylation was limited to IgG responses against the spike protein which is

expressed on the surface, whereas the pattern of core fucosylation remained largely intact at normal levels in IgGs formed against the viral nucleocapsid protein which lacks surface expression [4]. This is in alignment with other instances of afucosylated IgG formation in other disorders, where afucosylated IgG production is seen in response to only surface proteins of enveloped viruses and surface alloantigens of host blood cells such as platelets [2,4,8]. Therefore, the specific surface expression of the spike protein on the viral envelope and on the membrane of infected host cells is hypothesized to play a key role in inducing a pattern of low core fucosylation in certain individuals [8]. However, spike protein is found abundantly expressed on the surface membrane of various host cells in the respiratory tract during SARS-CoV-2 infection, raising the question of whether specific cell populations expressing spike protein may be required in order to induce such aberrant afucosylated responses that drive disease progression.

The body of knowledge in regards to afucosylated responses is currently limited. In Fetal and Neonatal AlloImmune Thrombocytopenia (FNAIT), afucosylated responses occur in reaction to alloantigens expressed on platelets' membrane and drive the immunopathology in this disorder [9].

A hypothesis emanating from this phenomenon would propose that the expression of spike protein on the surface membrane of platelets in COVID-19 may similarly induce formation of afucosylated anti-spike IgGs and drive the immunopathology of severe illness. While it is known that whole and infectious SARS-CoV-2 is found within platelets in severe COVID-19, multiple studies utilizing electron microscopy and immunogold labeling for the spike protein have found the spike protein to be limited only to the cytoplasmic lumen, and not expressed on the surface membrane of the infected platelets [3,4]. Bone marrow megakaryocytes (MKs) are, however, known to be infected by SARS-CoV-2 as well, and are believed to be the most likely source of infected platelets in COVID-19 [3,4]. In fact, infected MKs were shown to transfer viral antigens including the spike protein to emerging platelets during thrombopoiesis [2]. Moreover, infected MKs found early in the illness were predictive of disease progression to severe COVID-19 similar to the predictive nature of early afucosylated anti-spike IgGs [2,3]. Thus far, however, no links between these two predictive factors have been discovered.

Based on the above lines of evidence, a hypothesis was made that, although the spike protein is not found expressed on the surface membrane of platelets, spike expression on the surface membrane of platelet progenitors may occur during thrombopoiesis as infected MKs transfer viral material including the spike protein to budding pro-platelets. Spike expression on the surface of pro-platelets during thrombopoiesis may then trigger the formation of afucosylated IgG against the spike protein, similar to the afucosylated responses seen in reaction to platelet surface alloantigens encountered during/in the FNAIT disorder [9]. The resulting

afucosylated anti-spike IgGs may then drive the immunopathology of severe COVID-19 by inducing a potent pro-inflammatory cascade of cytokine production and FcγR-mediated stimulation of macrophages, monocytes, natural killer cells, and platelets. This hypothesis was put to test in this work.

Methods:

After obtaining informed consent and with the approval of the local ethics committee (No. PR-1113–22), megakaryocytes were isolated from the bone marrow of a cohort of (n=4) human subjects infected by SARS-CoV-2 who were experiencing severe COVID-19. Mean duration from the onset of severe disease to bone marrow biopsy was 3.2±0.85 days. The isolated megakaryocytes were transferred to an appropriate culture medium containing thrombopoietin and allowed to develop and differentiate under favorable growth conditions. The presence of SARS-CoV-2 was confirmed in the isolated MKs using a real-time PCR. Isolated MKs confirmed to harbor SARS-CoV-2 were then injected into K18-hACE2 transgenic mice (RRID: IMSR_JAX:034860). Mice were monitored for signs of infection, disease, or behavioral changes. Blood samples were taken to assess the response to injection of the mice with infected human MKs, and to quantify plasma levels of afucosylated IgGs and other molecules (serotonin and PF4) by ELISA. Once mice were euthanized, lung sections were prepared and processed with routine hematoxylin and eosin (H&E) staining and histopathological analysis was performed.

Results:

Pro-platelets derived from infected MKs express SARS-CoV-2 spike protein on their surface membrane during their maturation.

In Figure 1, the periphery of infected MKs-derived pro-platelets appears in green as labeled with anti-α-tubulin, while SARS-CoV-2 spike protein appears in red on the surface membrane of pro-platelets. Visualization was performed by confocal microscopy (teaser) after proplatelet fixation.

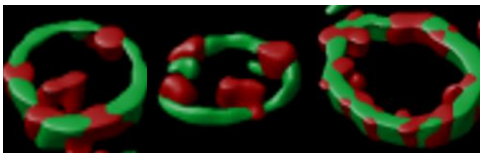

Figure 1. In green: α-tubulin that marks the periphery of proplatelets from MKs. **In red:** Spike S localized on the surface of pro-platelets

Injection of human MKs infected by SARS-CoV-2 into K18-hACE2 transgenic mice significantly promoted the formation afucosylated IgG antibodies against the spike protein (Figure 2).

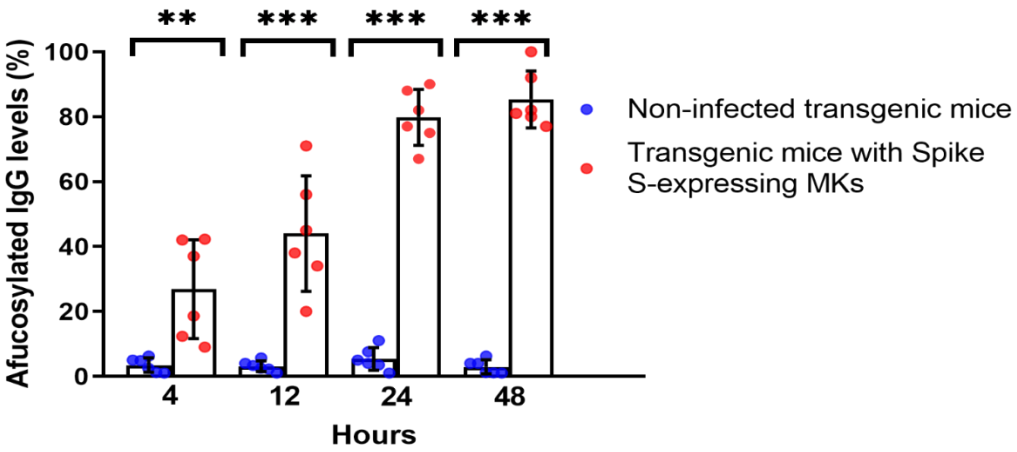

Figure 2: Mice infected with human megakaryocytes expressing the Spike S protein on their surface produce significantly elevated levels of afucosylated IgG antibodies.

These afucosylated IgG antibodies are pathogenic and contribute significantly to the severity of COVID-19.

The mortality rate in infected mice was proportional to the level of afucosylated IgG produced (Table 1).

Table 1: The mortality rate in mice injected with SARS-CoV-2-infected MK cells compared to non-injected mice.
Data are presented as mean ± standard deviation. Statistical analysis: unpaired Student's t-test was used to calculate p-values. Bold numbers indicate statistical significance at $p \leq 0.05$.

	Infected mice	**Non infected mice**	***p*-Value**
Mortality rate after 3 days (%)	33.3	8.3	**0.02**
Mortality rate after 7 days (%)	58.3	8.3	**0.01**

Taken together, these findings suggest that the incorporation of SARS-CoV-2 Spike protein into platelets during their maturation may play a critical role in the pathogenesis of COVID-19 by promoting the formation of afucosylated IgG antibodies, which can lead to more severe disease outcomes.

The plasma levels of PF4 and serotonin in the uninfected mice (marked in blue) are significantly lower than the levels of PF4 and serotonin in the infected mice (marked in red).

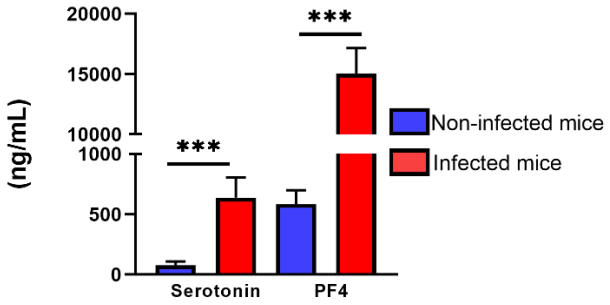

Figure 3: The plasma levels of serotonin and PF4 are significantly elevated in infected mice compared to non-infected mice

The first panel shows a control with normal vessel and lung tissue, whereas in the other cross-sections 4H and 24H post-infection of the transgenic mice with human megakaryocytes expressing Spike-S, invading inflammatory cells and thrombus in the vessel lumen and distortions of the lung tissue can be seen. Tissue visualization was performed using a microscope (20X) after Hematoxylin and Eosin staining.

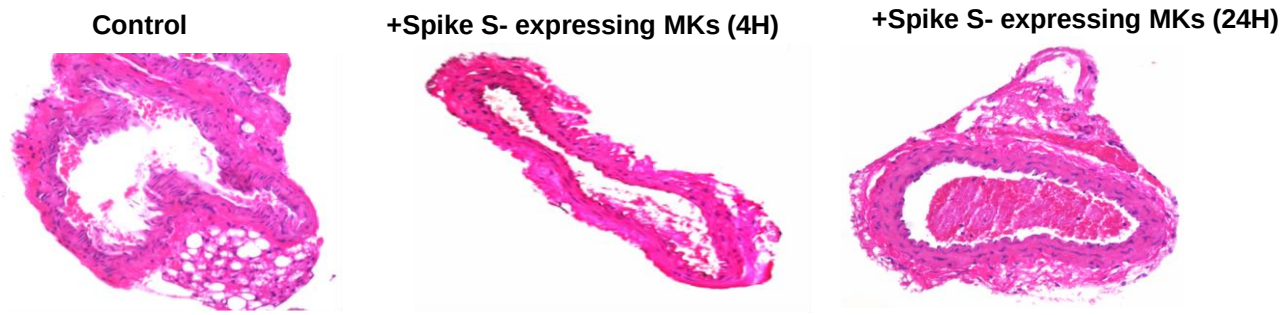

Figure 4: Infection of transgenic mice with human megakaryocytes expressing Spike-S induces the formation of pulmonary thromboembolism (MK: megakaryocytes).

Discussion:

Based on our investigation, it appears that the presence of Spike protein on the surface membrane of pro-platelets and platelets derived from virus-infected human megakaryocytes (MKs) could potentially lead to the production of pathogenic afucosylated IgG antibodies. This phenomenon is likely to occur within the bone marrow and lungs. The aberrant expression of Spike protein on pro-platelets' surface membranes could potentially be recognized by the immune system as a highly abnormal signal, which could potentially trigger the formation of highly potent afucosylated IgG antibodies specifically targeting Spike protein. Our study

suggests that there is a possibility of detecting significantly elevated levels of these antibodies in mice once challenged with SARS-CoV-2-infetcted human megakaryocytes.

The clinical manifestations of COVID-19 exhibit considerable heterogeneity, encompassing asymptomatic or mild self-limiting infections to severe airway inflammation culminating in respiratory distress, frequently resulting in fatality [1,10]. Notably, both extremes of disease trajectory share comparable initial responses, with patients experiencing a period of relatively mild symptoms lasting approximately one week. Subsequently, there is a dichotomy in outcomes, with either disease resolution or progression towards a highly exacerbated and potentially life-threatening phenotype [1,10]. The temporal discrepancy in the onset of these divergent responses, along with the contrasting clinical outcomes, implies distinct immunological pathways undertaken by the host immune system in its battle against the disease.

In general, antibodies play a pivotal role in host defense against infections, employing diverse mechanisms to counteract pathogens [11]. The effectiveness of these mechanisms is influenced by intrinsic characteristics of the antibodies, including isotype, subclass, allotype and glycosylation. In the context of critically ill COVID-19 patients, alterations in the glycosylation patterns of anti-Spike IgG antibodies have been observed [12]. These modifications amplify the effector functions of IgG, leading to heightened pro-inflammatory cytokine production [13,14].

Decreased fucosylation of IgG is known to enhance affinity for FcγRIII receptors [12]. Previous studies have demonstrated that SARS-CoV induces severe inflammation and lung damage mediated by IgG antibodies [15]. Utilizing a macaque model infected with SARS-CoV, Liu *et al*. demonstrated that the early emergence of anti-Spike-CoV IgG antibodies leads to pronounced lung injury by disrupting the polarization of macrophages, shifting them from a "M2" healing phenotype to a highly pro-inflammatory state [15]. Notably, patients with severe COVID-19 exhibit early and elevated titers of IgG antibodies [16–18], accompanied by a similar shift of anti-inflammatory lung macrophages towards a pro-inflammatory phenotype [19]. Collectively, these findings suggest the involvement of anti-Spike IgG antibodies in the development of severe COVID-19 cases.

In addition to the cytokine storm-induced pulmonary inflammation, severe forms of COVID-19 are characterized by the presence of pulmonary edema resulting from microvascular endothelial rupture [20], as well as coagulopathy leading to pulmonary immuno-thrombosis in many patients [21].

Therefore, the objective of this study was to investigate the hypothesis that, similar to the immune response observed in SARS, anti-Spike antibodies contribute to endothelial injury in severe cases of COVID-19. This mechanism could potentially explain the activation of platelets and subsequent release of alpha and dense granule contents such as FP4 and Serotonin.

These findings underscore the urgency to further investigate and elucidate the signaling pathways that initiate the generation of afucosylated IgG antibodies. Such understanding would enable the development of tailored vaccines that can elicit the desired fucosylated Fc antibody response. Moreover, it would facilitate the identification of therapeutic interventions aimed at preventing the formation of these pathogenic antibodies, which play a significant role in the morbidity and mortality associated with COVID-19, dengue fever, malaria, HIV, and other diseases.

Conclusion:

The proposed involvement of afucosylated antibodies in the pathogenesis of COVID-19 presents new therapeutic opportunities for addressing this disease. Future endeavors to generate high-titer COVID-19 immunoglobulins should consider utilizing plasma enriched with fucosylated anti-SARS-CoV2 antibodies. This approach could potentially replace the afucosylated anti-SARS-CoV2 IgG responses observed in patients, thereby preventing the worsening of symptoms and facilitating virus neutralization. Consequently, gaining a deeper understanding of the balance and regulation of afucosylated immune responses represents a captivating avenue for future research, with implications for advancing our comprehension of human immunity in both infectious and non-infectious conditions. Moreover, such insights could inform the development of enhanced vaccines that effectively mimic appropriate antigen presentation and environmental cues.

Data Availability Statement

The original contributions presented in the study are included in the article, and further inquiries can be directed to the corresponding author.

Ethical Approval Statement

Written informed consent was obtained from the individuals for the publication of any data included in this article.

Authors' Contribution

F. J., F. G. and Y. Z. designed the research. M. M., N. Z., A. N., F. G. and Y. Z. performed the research and statistical analyses. F. J., L. K., F. G., and Y. Z. analyzed the data. F. J., M. K., F. G. and Y. Z. contributed to discussion and revised the manuscript. F. J., M. M., F. G. and Y. Z. wrote the manuscript. All authors read and approved the paper.

Funding

This study was supported by Balvi Filantropic Fund (PR-BLV-20230125-B59).

Conflict of Interest

None declared.

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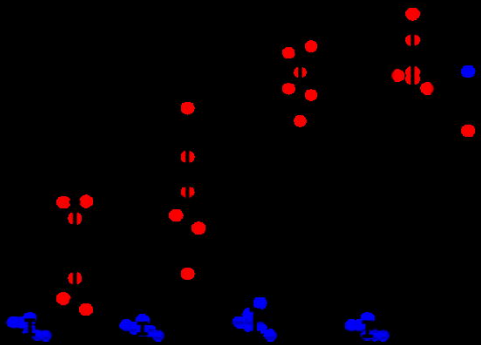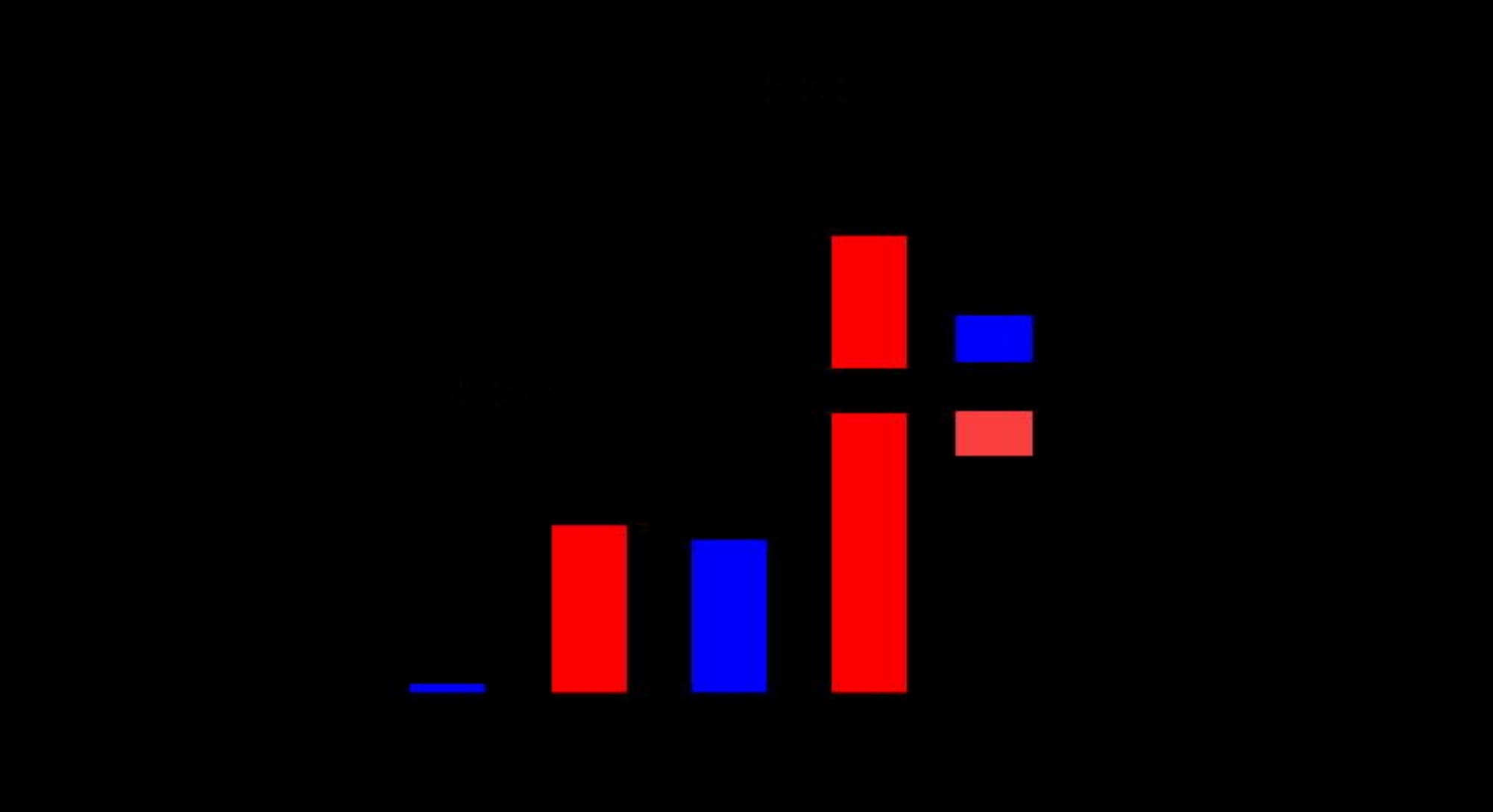
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