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PLATELETS AND THROMBOPOIESIS

Comment on Bye et al, page 1481

Sugar and spike: not so nice

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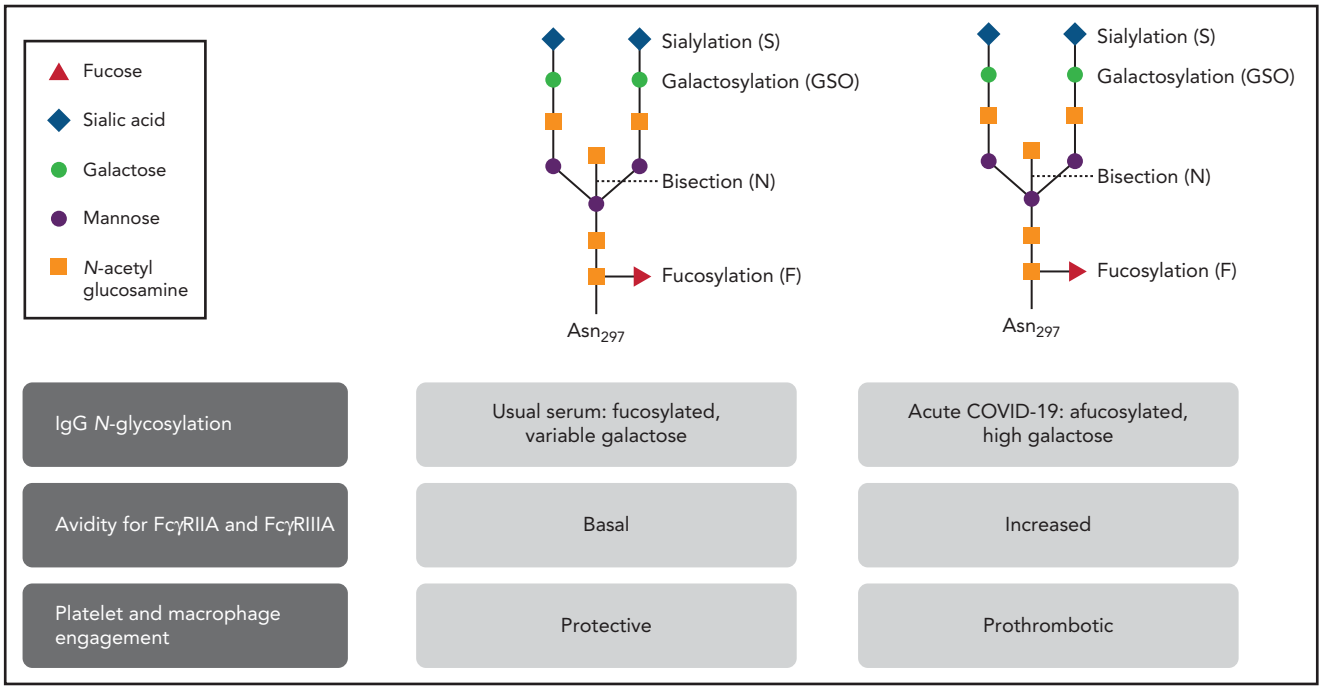
Bye and colleagues employed a new way to look at the thrombosis that accompanies COVID-19 infection.[1] In this issue of *Blood*, they have identified that aberrant glycosylation of the anti-spike IgG leads to greater prothrombotic platelet activation via FcγRIIA.

Over the past 2 decades, the interplay among immunity, inflammation, hemostasis, and thrombosis has been more clearly appreciated. Advances in fundamental immunology have translated into improved understanding of the relationship of human thrombotic disorders and immune stimuli. This work by Bye and colleagues fits nicely into that tradition. Thrombosis related to COVID-19 is important and incompletely understood. It is clearly multifactorial, with alterations of endothelial cells and activation of leukocytes, platelets, and coagulation. Since the beginning of the pandemic, clinicians have recognized that critical pulmonary (acute respiratory distress syndrome with pulmonary vascular thrombosis) and systemic (deep vein thrombosis) manifestations of COVID-19 infection are often pronounced when adaptive immunity has begun. IgG is an important component of the adaptive response. Immunoglobulin (IgG) undergoes *N*-glycosylation of the heavy chain in the Fc region during biosynthesis (see figure). The nature of these sugars in circulating IgG has been well studied, consistent with fucose and galactose residues being regulated in a narrow range. New fundamental studies have identified reduced fucosylation and increased galactosylation status of the IgG in response to certain viral infections, including HIV and dengue, and indicate that it may be a generalizable feature of the early IgG response to enveloped viruses that bud from cells.[2-4]

Enter the human IgG response to the COVID-19 spike protein. Bye et al build on the data of reduced fucosylation and increased galactosylation of anti-SARS-CoV-2 IgG directed against the spike protein to pursue the prothrombotic effects on



The *N*-glycosylation of IgG in severe acute COVID-19 infection has a variant pattern that has greater avidity for FcγRIIA and FcγRIIIA. As a result, platelet and macrophage engagement is prothrombotic. Professional illustration by Patrick Lane, ScEYEnce Studios.

platelets. A recombinant antispike IgG (Cova1-18) cloned from a patient[5] was modified to have usual glycosylation or low fucose (fuc) or high galactose (gal), or both. Only the low fuc/high gal IgG supported platelet accumulation over a von Willebrand factor (VWF)-coated surface in a flow chamber, at both venous and arterial shear rates. Bye et al decided to use the VWF-coated surface based on observations in vivo and ex vivo of high vWF on endothelial surfaces during COVID-19 infection. They show that prothrombotic platelet activation depends on interaction of the spike protein/Cova1-18 anti-SARS-CoV-2 IgG immune complex with FcγRIIa, the sole Fcγ receptor on human platelets that is capable of mediating immunity and thrombosis.[6] Additional evidence includes blockade of FcγRIIA with the anti-FcγRIIA ligand-blocking antibody IV.3 and reduction in activation with inhibitors to known platelet-signaling components downstream of FcγRIIA (Syk, Btk, and P2Y12).

Together with a related study of anti-SARS-CoV-2 IgG stimulation of alveolar macrophages,[4] Bye et al show that both FcγRIIA and FcγRIIIA have increased avidity for the IgG Fc end that presents low-fuc/high-gal modifications. The result is increased effector functions, which in this case are prothrombotic. This paradigm is a new one for antibody-dependent inflammation contributing to prothrombotic effects (see figure). The new appreciation of the interactions of underfucosylated/overgalactosylated IgG with Fcγ receptors could advance the understanding and management of COVID-19, coagulopathies from other enveloped viruses, and other immune thrombotic thrombocytopenic disorders.

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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Yanatori et al, page 1490

CD63 orchestrates ferritin export

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In this issue of *Blood*, Yanatori et al[1] demonstrate that the vesicular protein CD63 is regulated by iron and facilitates the secretion of iron-laden ferritin into extracellular vesicles, suggesting that CD63 may enable the transfer of iron-rich ferritin among cells.

Ferritin is a 24-subunit protein nanocage best known for its ability to store intracellular iron in a nontoxic but bioavailable form. A typical ferritin nanocage contains 1000 to 1500 atoms of iron and has the capacity to store >4000 atoms of iron, making it the principal intracellular iron reservoir and a critical contributor to the maintenance of intracellular iron homeostasis. However, ferritin is also found in extracellular compartments, notably human plasma. Levels of ferritin in plasma correlate with body iron stores and are also elevated in inflammation. Clinically, serum ferritin levels are used to assess iron status. The presence of ferritin in extracellular compartments has led to the suspicion that ferritin may play a role in extracellular as well as intracellular spaces, although the nature of that role and the mechanism by which ferritin reaches external environments have remained obscure for decades and are still incompletely understood.

A turning point in our understanding of extracellular ferritin came in 2018, when Truman-Rosentsvit et al[2] reported that ferritin can be secreted from macrophages through noncanonical pathways involving extracellular vesicles. However, what triggers ferritin secretion and how it is coordinated with intracellular iron metabolism remained unclear.

Yanatori et al now demonstrate that increased iron levels increase expression of CD63, a tetraspanin protein and important constituent of extracellular vesicles. Mechanistically, CD63 induction is accomplished through an iron responsive element (IRE) in the 5′ untranslated region of CD63 messenger RNA (mRNA) that is controlled by the same iron regulatory network that controls levels of ferritin itself. This network consists of iron regulatory proteins, which bind to IREs in the untranslated regions of selected mRNAs to posttranscriptionally control their activity or stability (see figure). Targets of the posttranscriptional iron regulatory network include not only ferritin but also proteins that regulate iron uptake and iron efflux, such as transferrin receptor 1 and ferroportin. Thus, the identification of a functional IRE in the 5′ untranslated region of CD63 directly connects CD63 to pathways of iron metabolism.

The authors further show that the induction of CD63 by iron has a functional impact on iron metabolism by increasing the secretion of $CD63^+$ extracellular vesicles containing iron-loaded ferritin. Mechanistically, iron loading stimulates