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EDITORIAL COMMENTARY

The good, the bad and the ugly: G-CSF, ageing and neutrophils—Implications for severe COVID-19

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Chronic inflammation associated with ageing, known also as inflammaging, contributes to and is associated with the pathogenesis of age-related diseases such as hypertension, arthritis, and diabetes.[1]

In the current issue of the *Journal of Leukocyte Biology*, He et al. report the observation that aged rhesus macaques contain fewer blood neutrophils despite higher levels of circulating Granulocyte colony stimulating factor (G-CSF), a major growth factor that drives neutrophil production in the bone marrow (BM).[2] Elevated G-CSF in older macaques was also positively correlated with higher levels of other proinflammatory cytokines such as IL-1β and MIF-1α. Furthermore, the authors observed that older macaques release neutrophils from the BM earlier than their younger counterparts and that these neutrophils are less functional. Some of these cells also possess a phenotype similar to polymorphonuclear myeloid-derived suppressor cells. This paradoxical decline in blood neutrophils despite elevated G-CSF and the observation that blood neutrophils within aged rhesus macaques are less functional may shed some light on how ageing increases the risk of runaway innate immune activation resulting in poorer infection outcomes.

Senescence of hematopoietic stem cells resulting from biological aging may cause a decline in blood neutrophil numbers resulting in a homeostatic response increasing G-CSF production to compensate. Increased G-CSF leads to shortened neutrophil maturation time in the BM and faster release into circulation. These rapidly produced but still immature neutrophils were shown in the accompanying report to express lower levels of the antimicrobial myeloperoxidase and perhaps they are less functional in other aspects such as abnormal trafficking and clearance from tissues or even perhaps the aberrant production of neutrophil extracellular traps (NETs).

Decreased neutrophil maturation time resulting in an immature, less functional phenotype may reduce the abundance of *good* (functional) neutrophils and provide the host with a *bad* set of less functional innate immune warriors that result in an *ugly* outcome in the event of an infection. The observations reported by He et al. likely provide a key piece to the current puzzle: why diabetics, persons of advanced age, and even obese individuals are at risk for severe COVID-19.[3] Both abnormalities in neutrophil function and dysregulation of a related cytokine, Granulocyte-macrophage colony stimulating factor (GM-CSF), appear to be common features in both diabetes and SARS-CoV-2 infection. Indeed, both impaired neutrophil function and increased GM-CSF have been associated with diabetes.[4] Similar observations have been made with regard to both G-CSF and GM-CSF and obesity,[5,6] and now in this report here, aging (ref). While it is already established that poor outcomes in SARS-CoV-2 infection involve excessive pulmonary neutrophil recruitment, accumulation, and formation of their extracellular traps in humans[7,8] and nonhuman primate studies,[9] the mechanism for the predisposition of persons with comorbid conditions associated with advanced age, diabetes, and obesity has not been adequately understood. Based on this new information, we propose a model (Figure 1) where under these conditions, SARS-CoV-2 infection results in recruitment of immature dysfunctional neutrophils. These cells are less able to clear debris while at the same time making the fight more difficult by aberrant NET production and accumulation due to a reduced capacity for proper trafficking. Patients with more severe COVID-19 exhibited elevated plasma levels of markers associated with NETs such as cell-free DNA.[10] The infection persists causing more inflammation and emergency granulopoiesis[11] further worsening the situation by flooding the lungs with even more immature dysfunctional neutrophils. Increased GM-CSF in the serum has been reported in type 2 diabetic subjects and is correlated with HbA1c levels.[4] In mice, obesity has been associated with elevated levels of inflammatory cytokines in a GM-CSF-dependent manner[5] and alter myeloid cell presence in the lung along with G-CSF.[6] In conditions, where GM-CSF and G-CSF are elevated, susceptibility to SARS-CoV-2 severe disease would be predicted. For example, exogenous G-CSF provided to cancer patients to prevent neutropenia and infection has been associated with increased need for supplemental oxygen and death.[12] This model emphasizes the role of GM-CSF and G-CSF as therapeutic targets for COVID-19 treatment. Indeed, blocking GM-CSF with a monoclonal antibody has been shown to improve severe COVID-19 outcomes in human subjects.[13] Other studies have shown that targeting G-CSF and its receptor (G-CSFR) with monoclonal antibodies can also mitigate neutrophil-dependent inflammatory responses.[14] A combinational approach in which both G-CSF and GM-CSF are targeted in severe COVID-19 may be even more effective. In our opinion, the findings reported by He and colleagues provide evidence for a link between aging and impaired neutrophil function that may help explained impaired innate immune cell function and resulting disease severity associated with severe COVID-19 infection.

In summary, He et al. report increased G-CSF levels in aged rhesus macaques that is associated with earlier neutrophil release from the BM. High G-CSF in these animals is associated with higher levels of

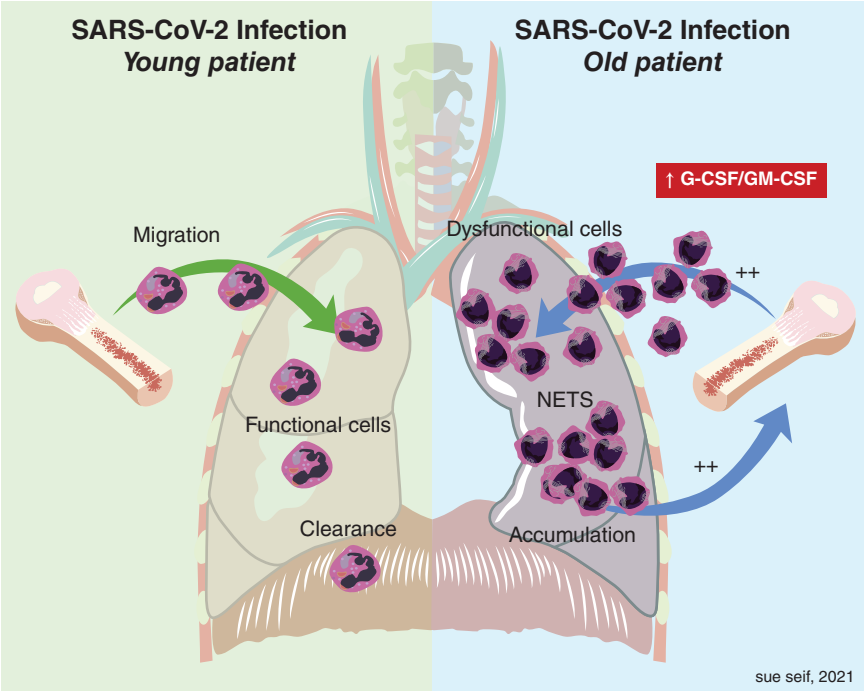

FIGURE 1 SARS-CoV-2 infection in young versus old. Elevated G-CSF and earlier neutrophil release may drive neutrophil pathology in severe COVID-19. In younger individuals, infection recruits mature neutrophils that function and are cleared appropriately. In older individuals, elevated G-CSF results in immature neutrophil release. Infection recruits these cells that may be less functional and thus accumulate and cannot clear as effectively. Infection persists and may drive further neutrophil migration and accumulation

other proinflammatory cytokines suggesting a serum cytokine signature of inflammaging. These early released neutrophils are less mature, functional, and some possess a unique myeloid derived suppressor cell phenotype. These findings have implications for the underlying causes of severe SARS-CoV-2 infection and resulting COVID-19 in the older human population and provide more evidence supporting the targeting of G-CSF and GM-CSF in the clinic.

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