LETTER TO THE EDITOR
Iran J Allergy Asthma Immunol
June 2022; 21(3):369-373.
Doi: 10.18502/ijaai.v21i3.9811

Do Low-density Granulocytes Induce Lymphopenia in Patients with COVID-19?

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Received: 7 December 2021; Received in revised form: 18 January 2022; Accepted: 29 January 2022

To The Editor

In December 2019, a new coronavirus disease (COVID-19), caused by a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged and spread rapidly to become a global epidemic or pandemic. Similar to other coronaviruses, the symptoms of the disease include fever, non-productive cough, dyspnoea, myalgia, and fatigue, with clinical manifestations of acute respiratory distress syndrome (ARDS).[1,2] The immune picture in the blood usually presents as neutrophilia and lymphopenia in most patients.[3] In addition, the neutrophil count positively correlates with disease severity while lymphocyte numbers are depleted in patients with worse clinical outcomes.[4] COVID-9 can be classified by severity into severe, moderate, and mild disease according to blood oxygen levels, respiratory rate, and the degree of lung involvement confirmed by radiology. Neutrophilia in extreme patients increases the ratio of neutrophils: lymphocytes (NLR) which correlate with high levels of D-dimer, more vascular thrombosis, and poor clinical outcomes.[3]

Data from several groups indicate increased numbers of $CD66b^+$ low-density neutrophils/granulocytes (LDNs/LDGs) within the peripheral blood mononuclear cell (PBMC) compartment of COVID-19 patients.[5-8] These blood LDGs are mainly granulocytic myeloid-derived suppressive cells or G-MDSCs and their levels are associated with COVID-19 severity.[5,9] Enhanced numbers of M-MDSCs were originally reported in the blood of patients with COVID-19 although this was not seen in endotracheal or nasopharyngeal aspirates.[10] M-MDSCs isolated from patients with COVID-19 attenuated the ability of T-cells to proliferate and release interferon (IFN)-γ. This process depended upon the activity of arginase 1 (Arg-1), elevated levels in COVID-19 patient blood.[11]

The number of circulating LDGs in severe and critically ill patients correlated with the raised levels of pro-inflammatory markers.[6,12,13] Subsequently, greater levels of LDNs that have intermediate CD16 ($CD16^{int}$) on their cell surface have been reported in the blood of patients with COVID-19, which were the primary immune cell found in bronchoalveolar lavage fluid (BAL).[13] These cells were functional in that they attenuated lymphocyte responses.[6,12,13] BAL LDNs

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exhibited transcriptomes with signatures reflecting proinflammatory pathways and functionally could activate platelets and form neutrophil extracellular traps (NETS) spontaneously and have an enhanced phagocytic capacity and cytokine production.[13] In addition, blood LDNs were associated with a reduced ability to degrade NETS.[12]

Four subtypes or states of blood LDGs were found in COVID-19 patients according to their surface marker expression reflecting different developmental stages[6], while transcriptomic analysis, reflecting altered functions or forms of granulocytic-MDSC (G-MDSC) indicates their importance in understanding COVID-19 severity. High levels of Arg1+ G-MDSC (Arg+G-MDSC) were found in the lungs and blood of COVID-19 patients who died. These cells are essential as they also express high levels of enzymes associated with reactive oxygen species generation and also suppress the function of T cells and endothelial cells. Interestingly, cells from COVID-19 patients expressed genes involved with pathways that included the production of Arg-1, degranulation of granulocytes, and granulocyte function, whilst those from asymptomatic patients expressed type I IFN-related genes.[14]

Notably, previous work had demonstrated that LDGs suppressed T and natural killer (NK) cell proliferation and function through their release of factors associated with COVID-19, such as Arg-1,[6] inducible nitric oxide synthase (iNOS)[15] and tumor growth factor-beta (TGFβ) (Figure 1).[16] In addition, autoantigens on LDGs are upregulated in COVID-19 patients suggesting an autoimmune component in severe disease.[7]

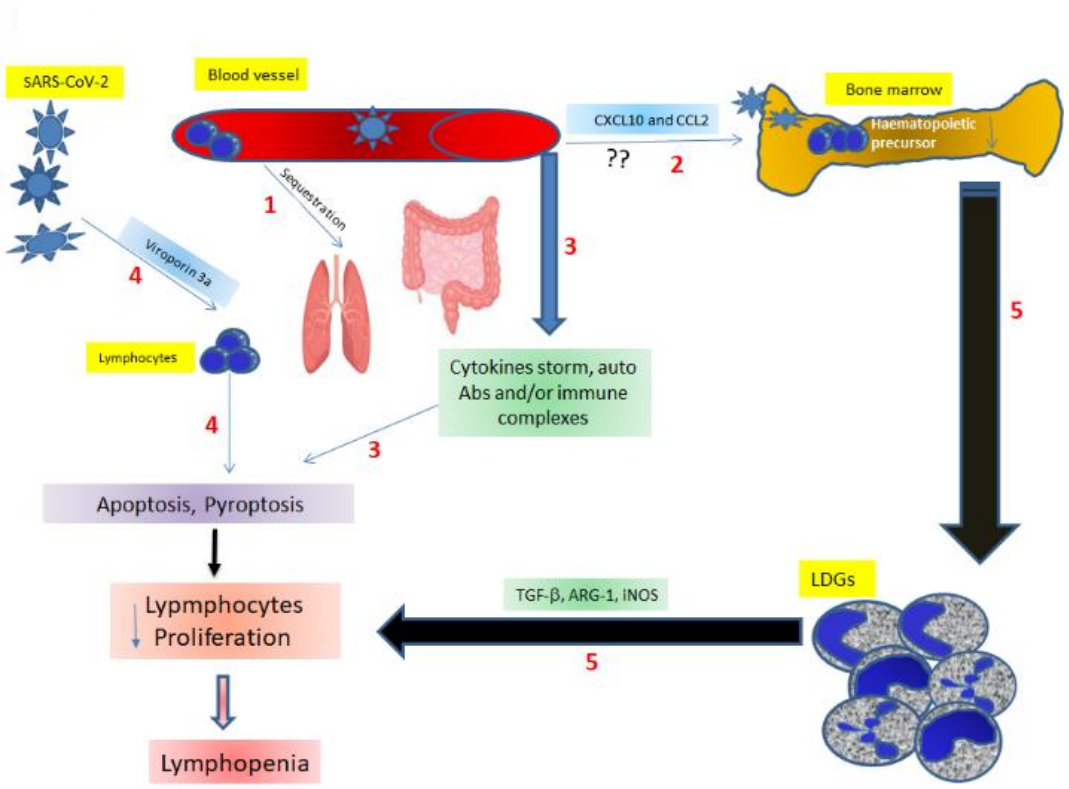

Figure 1. Schematic cartoon of the proposed mechanisms leading to lymphopenia in patients with coronavirus disease-2019 (COVID-19). The arrows with numbers indicate 1) Redistribution of lymphocytes in vital organs such as the lungs and intestine. 2) Possible suppressive effects of increased serum levels of chemokines such as CXCL-10 and CCL-2 on the hematopoiesis of stem cells in the bone marrow. 3) Induction of a cytokine storm, autoantibodies, and other immune complexes which cause lymphocyte apoptosis. 4) Indicated effect of virus-derived viroporin 3a on lymphocyte apoptosis and pyroptosis. 5) Possible role of mediators released from low-density granulocytes (LDGs) cells in suppressing lymphocyte proliferation.

LDGs describe a heterogeneous population of granulocytic myeloid cells isolated from PBMC after density gradient centrifugation. Different types of these cells exist with LDGs from cord blood for example containing $CD66^{hi}$, $CD33^{+}$, $CD14^{lo}$, and HLA-DR− markers.[9,17] Heightened numbers of LDGs have been reported previously in pathologic conditions such as cancer and psoriasis, as well as infectious diseases such as HIV infection and sepsis. In addition, elevated levels of LDGs are reported in a rat model of arthritis.[18-22] Moreover, LDG numbers may increase in conditions where cell activation due to chronic inflammation results in decreased granulocyte density.[17-19] Thus, LDGs present heterogeneous phenotypes or states in different diseases, possibly reflecting different stages in the maturity of these "neutrophil-like" cell populations.[17]

Increased cytokine levels of IL-8, granulocyte monocyte, and granulocyte–colony-stimulating factors (GM-CSF and G-CSF respectively) correlated with all LDG subsets in blood. These mediators are highly expressed in COVID-19 and there is an increased frequency of CD16+ LDGs in the blood of COVID-19 patients reflecting COVID-19 severity.[18] In contrast, the presence of CD16- LDGs is indicative of a recovery in severely ill COVID-19 patients.[6]

LDGs result from the activation and degranulation of mature normal density granulocytes, which have an immunosuppressive capacity.[17-19] Not surprisingly, LDGs in severe COVID-19 patients express genes related to an immunosuppressive phenotype[20], possibly associated with the lymphopenia seen in COVID-19 patients[21] This suggests that polymorph nuclear cell (PMN) activation and the resultant induction of LDGs are accelerated in COVID-19 patients. In addition, infection by SARS-CoV-2 induces immune cell apoptosis and pyroptosis[22,23] and the release of C-C motif and C-X-C motif chemokine ligands such as CCL-2 and CXCL-10, which, in turn, impairs hematopoiesis,[24,25] induces a cytokine storm and the generation of autoantibodies.[26,27] There is also a redistribution of lymphocytes within the liver and gastrointestinal tract[28] leading to lymphopenia in patients with COVID-19 (Figure 1).

Lymphopenia is the hallmark of the dysregulated immunity resulting from infection by SARS-CoV-2 and the expansion of LDG subsets, particularly G-MDSCs, requires additional study. It is important to further characterize LDGs and to define their role as markers of disease severity or as potential therapeutic targets in COVID-19.

In COVID-19 patients, circulating levels of cytokines, immunocomplexes, and/or autoantibodies modulate the bone marrow niche that allows the subsequent release of immature granulocytes especially LDGs into the circulation (Figure 1). However, it is unclear whether it is the cytokine storm or immune complexes that affect the morphology and gene expression profiles of bone marrow-derived granulocytes. Furthermore, since the LDG surface marker profile in COVID-19 is linked to cellular maturity, LDGs may be a sub-phenotype of activated neutrophils which are rare in healthy subjects.[29] Overall, direct and indirect observations indicated that LDGs are available in the blood of COVID-19 patients and their mediators such as TGF-β and arginase inhibit the proliferation of lymphocytes leading to lymphopenia. It is essential to clarify LDG origin, maturation status, and pathogenicity in murine models of SARS-CoV-2 models of autoimmunity. These models can test potential therapeutic agents that modulate these cells and suppress lymphocyte proliferation. Future characterization and establishment of specific LDG markers may also prove very useful in assessing their putative roles in other diseases where tissue damage occurs, such as lupus.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

ACKNOWLEDGEMENTS

Not Applicable.

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