

Importance of Efferocytosis in COVID-19 Mortality

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Abstract: COVID-19 is a generally benign coronavirus disease that can spread rapidly, except for those with a group of risk factors. Since the pathogenesis responsible for the severity of the disease has not been clearly revealed, effective treatment alternatives has not been developed. The hallmark of the SARS-CoV-2-infected cells is apoptosis. Apoptotic cells are cleared through a sterile process defined as efferocytosis by professional and nonprofessional phagocytic cells. The disease would be rapidly brought under control in the organism that can achieve effective efferocytosis, which is also a kind of innate immune response. In the risk group, the efferocytic process is defective. With the addition of the apoptotic cell load associated with SARS-CoV-2 infection, failure to achieve efferocytosis of dying cells can initiate secondary necrosis, which is a highly destructive process. Uncontrolled inflammation and coagulation abnormalities caused by secondary necrosis reason in various organ failures, lung in particular, which are responsible for the poor prognosis. Following the short and simplified information, this opinion paper aims to present possible treatment options that can control the severity of COVID-19 by detailing the mechanisms that can cause defective efferocytosis.

Keywords: COVID-19, SARS-CoV-2, efferocytosis: ADAM17, phosphatidylserine

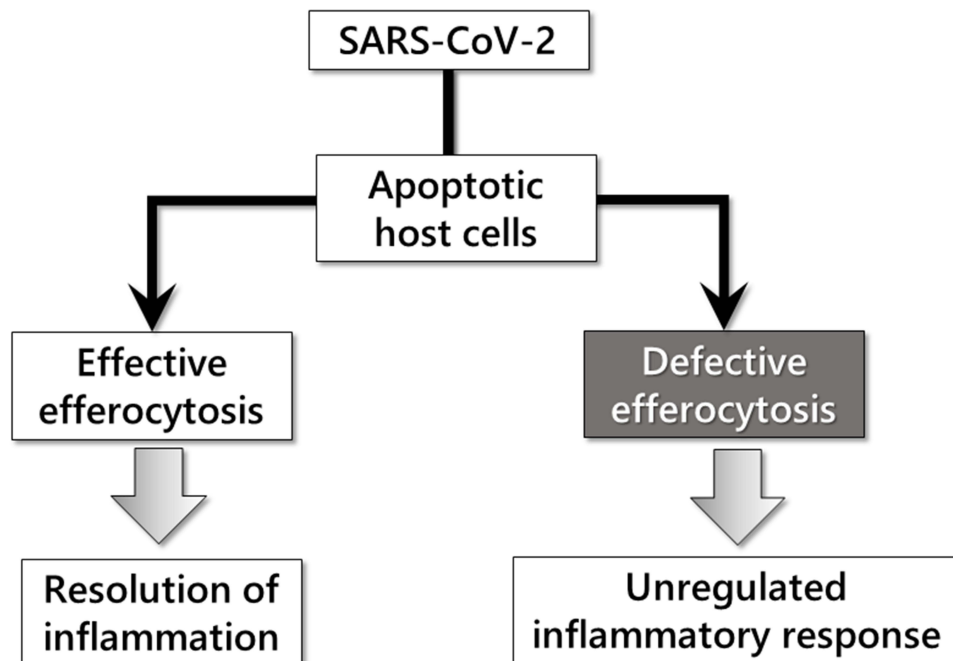
SARS-CoV-2-induced COVID-19 pandemic has been caused a serious increase in morbidity and mortality, as well as devastating problems in the economies of countries. Unprecedented rapid and widespread vaccination has provided significant relief in the control of the pandemic in a short period, less than a year. However, the belief that vaccines can solve everything has brought with it troubles. The dominance of the delta variant, the rapid increase in the number of cases and the rate of hospitalization despite vaccination necessitate taking new measures.¹ People infected with the delta variant of SARS-CoV-2 seem to be more likely to spread the virus before developing symptoms. More importantly, there was no difference in the rate of transmission and viral load between vaccinated and unvaccinated people, suggesting that a proportion of vaccinated people can transmit this variant.^{2,3} It is also a strong possibility that those who have been vaccinated are more contagious because they are asymptomatic or are easily accepted at social events. Moreover, SARS-CoV-2 lambda, a new variant of interest, exhibits higher infectivity and confers resistance to antiviral immunity.⁴ At least for these reasons, it seems difficult to control the pandemic with vaccination alone. While COVID-19 is threatening in a certain risk group, it generally shows a moderate course. Therefore, the development of treatment methods that can control the severity and lethality of the disease seems to be the most rational option since the beginning of the pandemic.

SARS-CoV-2 Infection and Efferocytosis

SARS-CoV-2 is an enveloped virus. Phosphatidylserine (PS) is expressed in the outer leaflet of the viral envelope, with a symmetrical location rather than the asymmetrical phospholipid distribution observed in the normal plasma membrane.⁵ As with all enveloped viruses, this facilitates cell entry of SARS-CoV-2 using the apoptotic mimicry pathway.⁶ PS receptors (PSRs), Axl in particular, were shown to potentiate the binding and uptake of SARS-CoV-2.⁶

SARS-CoV-2 spike protein (S) is divided into two subunits: S1 unit of S binds to angiotensin-converting enzyme 2 (ACE2), while S2 mediates membrane fusion. Several arginine residues present in SARS-CoV-2 S are cleaved by the host protease, furin that is not found in SARS-CoV-related coronaviruses.⁷ Proteolytic processing of SARS-CoV-2 S in the virus-producing host cells, rather than during entry into target cells, may facilitate subsequent binding to the ACE2 receptors, fusion, and entry into target cells.⁸ Accordingly, loss of the furin cleavage site has been shown to affect the

Graphical Abstract



pathogenesis of SARS-CoV-2, suggesting that the furin cleavage site has a critical role in the transmission of SARS-CoV-2 infection.⁹

SARS-CoV-2 genome contains 29 open reading frames (ORFs), which encode 29 proteins. Among these, the ORF3a protein is an accessory protein specific to SARS-CoVs. ORF3a is involved in critical steps of the viral infection cycle and determines viral replication and virulence of SARS-CoV-2.¹⁰ In cells infected with SARS-CoV-2, the virus enforces the apoptotic process through ORF3a.¹¹ ORF3a-mediated caspase-3 activation initiates both the apoptotic process and a series of changes for the rapid elimination of the apoptotic cell by the phagocytic system:

1. Phospholipase A2 activation increases the level of lysophosphatidylcholine on the outer membrane surface.¹²
2. The opening of membrane Pannexin channels causes an increase in extracellular ATP, UTP, and spermidine levels.¹²
3. An increase in oxidized PS and phosphatidylcholine levels occurs.¹³ All of these three pathways act as “find me” signals for the phagocytic cells.
4. Irreversible activation of Xkr8, a membrane scramblase, causes PS to be expressed in the membrane outer leaflet, which is the essential “eat me” signal for cells of the phagocytic system.^{14,15}

In a healthy organism, professional and non-professional phagocytic cells detect “find me” and “eat me” signals and rapidly clear apoptotic cells without damaging the environment through a process known as efferocytosis.^{16–18} While macrophages, neutrophils and dendritic cells originating from the bloodstream are professional; local epithelial, endothelial, and fibrotic cells are the elements of the non-professional phagocytic system.¹⁹

A large number of different PSRs that recognize PS directly and indirectly in phagocytic cells facilitate the functioning of the efferocytosis process.^{5,6,14} While TIM receptors (T cell immunoglobulin and mucin domain-containing molecules) provide direct binding, TAM (Tyro3, Axl, and MerTK) receptors indirectly bind to apoptotic cells via

mediator molecules, such as Gas6 and protein S (ProS).^{5,18} Among several other PSRs, the importance of the phagocytic cell scavenger receptor CD36 in engulfing the apoptotic cell should be particularly emphasized.²⁰

Cells exposed to apoptotic environments undergo conformational and biochemical changes in their membranes. The hydrolysis of phosphatidylcholine to lysophosphatidylcholine renders the phosphatidylcholine group accessible for the binding of C-reactive protein (CRP). CRP irreversibly marks apoptotic cells for the activation of the classical complement pathway, ultimately those opsonized cells are disposed of by phagocytosis.^{21,22} Apoptotic cells tagged with CRP may also interact with the scavenger CD36 receptors, which are expressed by the macrophages and thus may facilitate efferocytosis.²³ In other words, CRP can promote efferocytosis by binding to the apoptotic cell surface, leading to recognition and phagocytosis by macrophages.²⁴ Consistently, CRP apheresis has been reported to be an effective treatment option for patients severely challenged by SARS-CoV-2 infection, most likely by regulating efferocytosis.^{25,26}

Efficient phagocytosis of apoptotic cells, efferocytosis, is one of the most essential components of maintaining tissue homeostasis. After engulfment of the apoptotic cell, phagocytic cells actively control inflammation (“tolerate me”) by releasing anti-inflammatory cytokines such as transforming growth factor-beta (TGF- β) and IL-10.¹⁴ This specialized phagocytosis of apoptotic cells also accelerates the presentation of viral antigens to lymphocytes and T cells, enhancing the activation of antigen-specific B and T cells.^{27,28}

If the efferocytosis-mediated clearance process is disrupted, the apoptotic cells undergo secondary necrosis. This is a highly detrimental process that results in leakage of toxic intracellular antigens, tissue destruction, and intensification of the existing inflammation.^{14,29} In secondary necrosis, increased neutrophil pro-inflammatory activity and excessive degranulation may cause acute respiratory distress syndrome (ARDS) or multiple organ failure as a result of neutrophil-mediated systemic inflammatory response syndrome.^{30,31} Membrane rupture due to secondary necrosis triggers the development of a different type of autoimmune response to auto-antigen release with auto-reactive CD4⁺ cell activation.³² Thus, efferocytosis, which means rapid and effective non-inflammatory and non-immunogenic apoptotic cell clearance through the interrelated “find me”, “eat me”, and “tolerate me” pathways, performs a unique function in the maintenance of homeostasis at the tissue and system level.

Collectively, COVID-19 patients with a relatively healthy premorbid history recover following a mild-to-moderate course through the functioning of the following mechanisms (Figure 1).

1. SARS-CoV-2 replication may lead to the pro-apoptotic changes that would trigger the exposure of normally intracellular anionic phospholipids, PS in particular, on the outer leaflet of the membrane surface of virus-infected cells. These virus-induced apoptotic cells need to be rapidly and efficiently removed otherwise they may produce secondary necrotic debris that promotes the release and hyperactivation of pro-inflammatory molecules from the immune effector cells.
2. Efficient efferocytosis is an essential component of tissue homeostasis and the resolution of inflammation. SARS-CoV-2-infected apoptotic cells expose PS as an “eat me” signal, which distinguishes them from live cells, initiates efferocytosis by professional and non-professional phagocytic cells in a non-inflammatory manner. After engulfing an apoptotic cell, macrophages actively dampen inflammation by releasing anti-inflammatory cytokines, such as (TGF- β) and interleukin-10, as well as pro-resolving lipid mediators for the resolution of inflammation.
3. SARS-CoV-2-infected, apoptotic cells are phagocytosed and digested together with the virus itself through the actions of degrading enzymes that exist in lysosomes of phagocytes, leading to the inhibition of viral growth. This mode of phagocytic elimination of invading pathogens may be considered as a cellular innate immune response.³³ Consistently, binding and preventing PS-exposing cells from being phagocytosed by annexin V, which binds specifically to PS, has been reported to augment the rate of mortality among virus-infected mice.³⁴
4. Under homeostatic conditions, anti-inflammatory cytokines and natural regulatory T cells channel dendritic cell antigen presentation to induce regulatory T-cell differentiation from naïve CD4⁺ T-cell precursors and to tolerize effector CD8⁺ T cells.³⁵ Furthermore, efferocytosis of infected apoptotic cells can help to mount an adaptive immune response against the virus through the process of antigen cross-presentation.³⁶

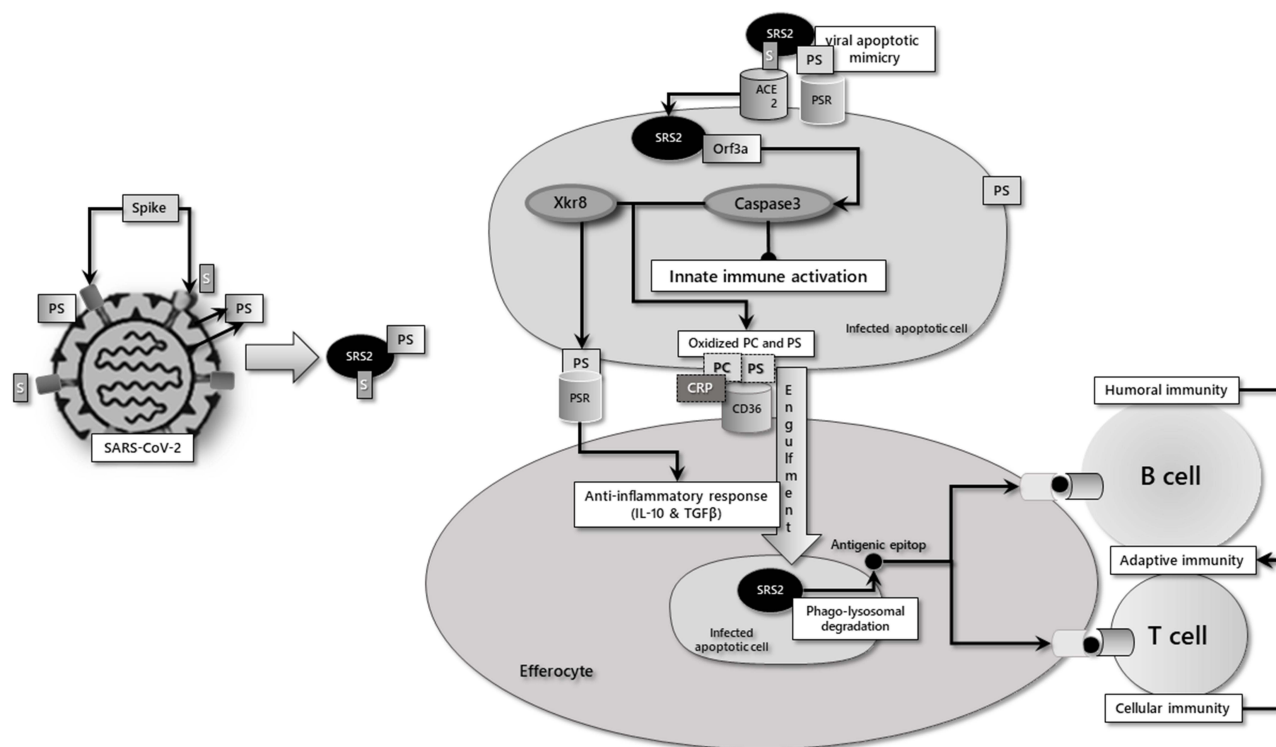


Figure 1 Simplified depiction of the apoptotic/efferoctytic process in SARS-COV-2 infection.

Notes: The phosphatidylserine (PS) in the envelope of the virus attaches to the PS receptors (PSRs) on the target cell, facilitating the binding of the viral spike protein to the host ACE2 receptor. In the cell, the viral ORF3a protein initiates the apoptotic process by the caspase-3 activation. While activated caspase-3 inhibits cellular innate immune response, caspase-3-driven permanent activation of the Xkr8 scramblase causes PS expression in the membrane outer leaflet of apoptotic cells. Caspase-3 also oxidizes PS and phosphatidylcholine (PC). PS binds to efferoctytic cells via PSRs, facilitating the binding of oxidized PC-CRP and PS to CD36 and engulfment of the apoptotic cell. Successfully induced efferoctytosis is a sterile process with the secretion of anti-inflammatory mediators. In addition, antigenic viral epitopes, which arise due to phago-lysosomal degradation of the virus in antigen-presenting cells, can initiate T- and B-cell-mediated adaptive immunity. Reproduced from Erol A. Defective efferoctytosis as a predictor of COVID-19 mortality. *OSF Preprints*. 2021; September 13:1-18.¹¹⁹

Thus, while COVID-19 is highly contagious due to the multibasic furin cleavage site in the S protein,³⁷ it follows a self-limiting course through the operation of the mechanisms briefly described. However, the disease may progress to a more severe and even fatal stage in those with chronic degenerative and cardiovascular diseases, especially in the elderly. If the underlying pathogenesis of this adverse progression could be demonstrated, it may also provide possible target(s) for the treatment.

Risk Factors for COVID-19 and Defective Efferoctytosis

Despite the death of billions of cells every day, effectively functioning efferoctytosis maintains normal physiology in the majority of the population; however, it becomes defective in unresolved chronic inflammatory diseases, causing apoptotic cell accumulation.³⁸ Diseases with increased morbidity and mortality, such as aging, obesity, heart diseases, and diabetes, cause an immuno-lipidomic imbalance with lipidome disorder and chronic inflammatory pathologies.^{39,40} The characteristic determinants of these disorders are low-level chronic inflammation and increased apoptotic cell load.⁴¹ Altogether, defective efferoctytosis is an essential pathology responsible for the development and progression of chronic inflammatory diseases, including atherosclerosis, obesity, diabetes, heart failure, chronic lung disease, and neurodegenerative disease, as well as cancer.⁴²

The common feature of most of these degenerative chronic disorders is increased ADAM-17 (a disintegrin and metalloproteinase 17) activity in most of the cells, including bronchial epithelial cells, endothelial cells, and phagocytic cells.^{43,44} ADAM-17, also known as TNF α -converting enzyme (TACE), is a sheddase, capable of proteolytic cleavage and release of membrane-bound molecules.^{45,46} Studies revealing the relationship between ADAM-17 and a group of

inflammatory degenerative diseases, including cancer, are reported with increasing frequency. Interestingly, transcriptional ADAM-17 protein expression has been observed to increase in diabetic subjects through the hyperglycemia-induced HIF-1 activity.^{47,48} In addition, the euglycemic effect of insulin restored ACE2 and ADAM-17 expression to the physiological levels,⁴⁷ suggesting that insulin may work as an ADAM17 inhibitor.⁴⁹ Furthermore, evidence is provided that ADAM-17 plays a role in metabolic syndrome-related pathologies.^{50,51} On the other hand, some clear data also show the possible relationship between the presence of metabolic syndrome and the severity of COVID-19.⁵² Accordingly, several of the features of the metabolic syndrome seem to be associated with a worse prognosis in patients with COVID-19.⁵³

ADAM-17 is the main secretase responsible for ectodomain shedding of most of the cell surface and the transmembrane proteins. At least 90 substrates are processed by ADAM-17. Its activity towards TNF α and IL-6R α could potentiate the proinflammatory effects of the cytokines.⁵⁴ In addition, ADAM-17 activity promotes lymphocyte and neutrophil translocation to the inflammatory sites.⁵⁵ Taken together, premorbid patients with increased ADAM17 and thus pro-inflammatory activity, if contracted SARS-CoV-2 infection, would have the inflammatory process more severe.⁵⁶

PS translocation to the membrane surface, an exclusive marker of apoptosis, is also required for the sheddase function of ADAM-17. The externalized PS in the membrane interacts electrostatically with ADAM-17 and then enables substrate processing to take place.⁵⁷ Signaling pathways destined to activate ADAM-17-sheddase function converge at the final step leading to scramblase activation with a breakdown of phospholipid asymmetry and exposure of PS at the outer leaflet.⁵⁸ On the other hand, PS surface exposure is required for the regulation of cholesterol metabolism, inflammation, and hemostasis.⁵⁹ Recalling that anti-inflammatory cytokines are secreted during PS-induced efferocytosis, it is possible to consider this as an effort to control the existing inflammatory pathology.⁶⁰ Therefore, exposure to PS on the outer plasma membrane is increased in chronic inflammatory disorders, including atherosclerotic diseases and diseases associated with coagulation disorders,^{59,61} which may potentiate the activity of ADAM-17.

In principle, all-natural membranes may function as a procoagulant phospholipid surface, if sufficient PS is present in the exofacial leaflet.⁶² Microparticles and microvesicles refer to large extracellular vesicles, which are small membranous structures.⁶³ Microparticles are present in the blood of healthy individuals, but their number is considerably increased in diseased states, such as inflammation, cardiovascular events, and cancer, where they may contribute to the increased risk of thrombotic events in these patients.⁶² Circulating microparticles are shed from the plasma membrane of a variety of cells, including platelets, erythrocytes, leukocytes, endothelial cells, and tumor cells as a result of activation and/or apoptosis.^{62,64} These microparticles have lost lipid asymmetry and expose PS, thus providing a procoagulant surface.⁶² The prothrombotic state mediated by PS-exposing microparticles is implicated in the adverse progression of chronic inflammatory diseases, including atherosclerosis.⁵⁹ In addition, ADAM-17 was shown to present in microparticles released from activated cells; thus, ADAM-17 activity can be distributed to more distant cells.⁶⁵ Remarkably, increased levels of microparticles in the circulation of severe COVID-19 patients that trigger the risk of thromboembolic complications were reported.⁶⁶ Furthermore, the level of PS-exposing microparticles correlated more strongly with the severity of COVID-19 than indices, such as lymphopenia, IL-6, D-Dimer, fibrinogen, and other established laboratory parameters.⁶⁷

ACE2, a metallopeptidase and a key component of the renin–angiotensin system (RAS), is the main receptor for the cellular entry of SARS-CoV and SARS-CoV-2. The binding of SARS viruses to ACE2 triggers the viral conformational changes, which may increase the proteolytic digestion between the S1 and S2 subunits.^{68,69} Indeed, ACE2 cleavage by ADAM-17 is required for efficient SARS-S-driven entry. Furthermore, ADAM-17-mediated shedding of ACE2 results in the release of the ectodomain into the circulation, which preserves its catalytic and bioactive potency, and the binding site for SARS-CoV-2.⁷⁰ Recent studies demonstrated that ADAM-17-driven soluble ACE2 (sACE2) levels are elevated in COVID-19 patients. The binding of SARS-CoV-2 by sACE2 may enable cell entry of tissues where membrane-bound ACE2 (mACE2) is poorly expressed.^{71,72} Low expression levels of mACE2 and associated higher ADAM-17 activity have been related to cardiovascular diseases. Consequently, the activity of the sACE2 is increased in patients with cardiovascular disease and hypertension, which correlates with the severity of the disease.⁷³ Thus, chronic diseases with increased membrane PS exposure and associated ADAM-17 activity may adversely affect the prognosis of the COVID-19 through the facilitated cellular virus entry as a result of the increased sACE2 levels.^{56,71}

PSRs, including TIM1, TAM, and CD36, can also be proteolytically cleaved by the transmembrane protease ADAM-17.^{20,74} This shedding can prevent receptor functions in macrophage-mediated apoptotic cell engulfment.⁷⁵ The soluble cleavage products may function as sequester ligands and decoy receptors, which can act as antagonists for those PSRs.^{75,76} Accordingly, the deletion of ADAM-17 has been shown to increase macrophage-mediated efferocytosis in vivo, resulting in an enhanced anti-inflammatory response.⁷⁷

Programmed cell death 1 (PD-1), a surface molecule and a member of the immunoglobulin superfamily, functions as co-inhibitory receptors during immune responses against pathogens and cancer. Hence, PD-1 is important in the regulation of the magnitude and quality of T cell response.⁷⁸ PD-1 interacts with the ligands PD-L1 and PD-L2. PD-L1 is expressed in all hematopoietic cells and also by many non-hematopoietic cell types, such as endothelial and epithelial cells. PD-L2 expression, on the other hand, is limited to hematopoietic cells, such as dendritic cells, B cells, and monocytes/macrophages.^{78,79} During the acute phase of virus infection, stimulation of the PD-1/PD-L1 signaling pathway modulates the strength and quality of cytotoxic CD8⁺ T cell attack, maintaining a balance between virus elimination and tissue damage.⁷⁸

ADAM17 can cleave PD-L1 from the surface of vesicles and cells.⁸⁰ Although circulating soluble PD-L1 (sPD-L1) has been largely known as a prognostic biomarker for cancers,⁸¹ sPD-L1 has also been detected in various pathologies, often associated with markers of inflammation.⁸² Of note, a recent study suggests that an increase in sPD-L1 may be a sign of poor prognosis as a result of dysregulation of the PD-1/PD-L1 axis in COVID-19 patients.⁸³

To summarize, the following mechanisms due to the increased PS exposure and ADAM-17 activity adversely affect the prognosis of the disease in those who have comorbidities along with the COVID-19 disease (Figure 2).

1. The proinflammatory burden that will determine the severity of COVID-19 is already at high levels before the onset of the disease.
2. ACE2 cleavage and sACE2 levels are increased. The facilitated extracellular association between sACE2 and SARS-CoV-2 may reinforce the infection more widespread and devastating.

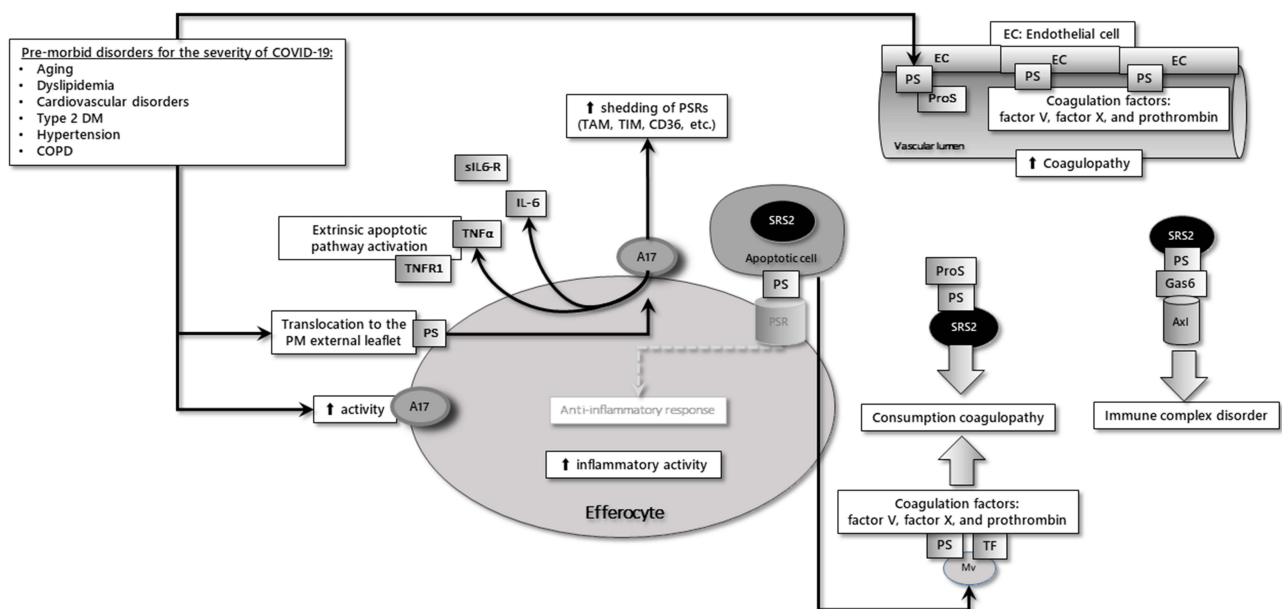


Figure 2 The common characteristics of co-morbid diseases responsible for the severity of COVID-19 are increased PS expression and ADAM-17 (A17) activity in cells. **Notes:** ADAM-17 renders efferocytosis as defective due to the shedding of the receptors required for the engulfment of apoptotic cells. PS expressed on the surface of endothelial cells, PS in the SARS-CoV-2 envelope, and microvesicles (Mv) detached from apoptotic cells reason in coagulopathy seen in COVID-19. Reproduced from Erol A. Defective efferocytosis as a predictor of COVID-19 mortality. *OSF Preprints*. 2021; September 13:1-18.¹¹⁹

3. Circulating microparticles have been implicated in cellular signaling processes in immune responses, inflammation, and coagulation. In addition to PS exposure on their membrane, they may contain ADAM17, which can contribute to substrate shedding on more distant cells.⁶⁵
4. Loss of PS receptors on phagocytic cells (efferocytes) impairs the recognition and engulfment process of apoptotic cells, thereby impairing efferocytosis.
5. Lastly, decreased expression of PD-L1 on T cells may reflect the induction of hyperactivation status, which may cause an excessive immunopathology.

Collectively, potentiated dysregulation of ADAM-17 activity may lead to the chronic operation of defective efferocytosis. In turn, defective efferocytosis accompanying several non-resolving, chronic inflammatory diseases may lead to the accumulation of dead cells. The dead cells can become secondarily necrotic, which can lead to tissue necrosis, autoimmunity, and pathological inflammation. Thus, defective efferocytosis is emerging as a key mechanism driving the development and progression of chronic inflammatory diseases, including atherosclerosis, obesity, diabetes, heart failure, chronic lung disease, neurodegenerative disease, and cancer.⁴²

Defective Efferocytosis and COVID-19 Severity

Efferocytosis is a mechanism for eliminating pathogens and pathogen-infected cells in an immunologically silent manner. The quality of efferocytic clearance of virus and virus-infected apoptotic cells depends on the virus load and the operation of efferocytic machinery.¹⁷ Therefore, increased viral load and defect in efferocytic cells seem to be the crucially important factors that will determine the prognosis of the disease. Patients in the risk group who already have problems in apoptotic cell clearance due to defective efferocytosis cannot cope with the additional acute apoptotic burden that occurs with COVID-19 disease. Consequently, the intractable inflammation that increases with secondary necrosis causes destruction and inevitable organ failure. Support for this approach came from a recent study showing that dysfunctional efferocytosis is the leading cause of the increased inflammatory response and widespread tissue damage seen in COVID-19.⁸⁴

Older age, obesity, diabetes, cardiovascular diseases, and active cancer have each been found to be independently associated with a higher mortality risk for COVID-19.⁸⁵ Furthermore, COVID-19 does not spare young people as well. The majority of comorbidities that play a role in the prognosis of the elderly have also been shown to be risk factors that may be responsible for the severity of COVID-19 for the young adult population aged 16–28 years.⁸⁶

Novel Treatment Options Targeting Defective Efferocytosis in COVID-19

The clinical trial research has occurred at an unprecedented pace during the COVID-19 pandemic. Among the many potential treatment options for COVID-19, only a few are effective and predictable for the clinical treatment of patients. Some immunomodulatory drugs such as anti-TNF, anti-IL-1, anti-IL-6 used in COVID-19 patients have been shown to support recovery, possibly by neutralizing the increased cytokine levels by the augmented ADAM17 activity.⁸⁷ In a study showing the effect of glucocorticoids, another group of immune modulators, dexamethasone treatment in hospitalized COVID-19 patients provided lower mortality among those requiring respiratory support.⁸⁸

Glucocorticoids are powerful anti-inflammatory agents by their ability to inhibit the recruitment of inflammatory cells and to downregulate proinflammatory cytokine expression.⁸⁹ Glucocorticoids have been shown to modulate the expression of over 100 genes, including those known to be associated with efferocytosis, such as Mer tyrosine kinase (MerTK), a member of TAM family PS receptors.⁹⁰ Dexamethasone was also shown to promote the expression of two PS-binding opsonins, protein S and MFG-E8, which facilitate MerTK-dependent efferocytosis of apoptotic cells.^{89,91} Thus, corticosterones, which provide limited support for defective efferocytosis, find wide use in clinical treatment as an effective drug that can control the severity of cases.

Peroxisome proliferator-activated receptor γ (PPAR γ) agonists such as pioglitazone alter cellular metabolism of the liver, adipose tissue, and muscle cells, as well as inflammatory macrophages. In addition, they have many anti-inflammatory effects including the suppression of Th1 and Th17-induced inflammation, inhibition of NF κ B, and upregulation of IL-10.^{92,93} Interestingly, PPAR γ agonists enhance MerTK and opsonin expression by human

macrophages, similar to glucocorticoids.⁹⁴ Furthermore, treatment with pioglitazone, either prophylactically or during inflammation, while significantly enhancing PPAR γ -mediated programming of efferocytes, reduces the accumulation of apoptotic cells.^{92,95} Therefore, low-dose pioglitazone alone or combination with glucocorticoids seems to be a readily available inexpensive, and effective treatment option for the correction of defective efferocytosis.^{93,96}

While ADAM-17 plays a crucial role in the pathogenesis of defective efferocytosis, it was shown that ADAM-17 activity is also important in the development and worst prognosis of COVID-19.⁹⁷ Therefore, inhibiting ADAM-17 activity seems to be a rational option in severe cases of COVID-19. Experimental evidence has been shown that the inhibition of ADAM-17 protects against lung inflammation by reducing cytokine storm and excessive neutrophil recruitment to the lung in a mouse model associated with COVID-19.⁹⁸ However, ADAM-17 has a broad regulatory role in human biological processes. Consistently, dramatic disorders affecting the intestines, lungs, eyes, and hair of ADAM-17-deficient mice and humans have prompted caution regarding therapeutic interventions for ADAM-17 blockade in humans.^{99,100} Nevertheless, researchers are nowadays focusing on developing strategies for selective inhibition of ADAM-17. Several compounds have recently been entered into clinical trials but have been withdrawn due to emerging adverse events.⁹⁷ Therefore, despite the central importance of ADAM-17 in the pathogenesis of the disease, the option of inhibiting ADAM-17 in severe cases of COVID-19 should be viewed with caution.

It has been emphasized above that the level of sACE2 in the circulation increases due to defective efferocytosis in diseases that constitute a risk group for COVID-19. However, another therapeutic approach proposes using sACE2 to trap and neutralize SARS-CoV-2. From this perspective, while mACE2 may mediate cell entry of SARS-CoV-2, a genetically modified soluble form of ACE2, called human recombinant soluble angiotensin-converting enzyme-2 (hrsACE2), may decrease cell entry of SARS-CoV-2 competing for mACE2.¹⁰¹ However, the plasma RAS balance in COVID-19 patients was already characterized by a strong transient increase in circulating plasma sACE2.⁷² Therefore, in COVID-19 patients with comorbid diseases, both Angiotensin II and virus adversely affect the prognosis by affecting the RAS balance in a way that down-regulates ACE2.¹⁰² Interestingly, a recent study reports encouraging results in the first severe COVID-19 patient treated with hrsACE2.¹⁰³ However, sACE2 concentrations used in experimental studies are much higher ($\mu\text{g/mL}$) than the physiological range of sACE2 concentrations (ng/mL). While a high range of sACE2 concentrations can compete with the SARS-CoV-2-ACE2 complex for cell entry, the physiological range of sACE2 may facilitate virus entry and thus SARS-CoV-2 infectivity. Therefore, a treatment that may alter sACE2 levels in the physiological range in patients with COVID-19 should be carefully considered.⁷¹

In severe cases of COVID-19 where defective efferocytosis is essential in the pathogenesis, the characteristic feature of infected-apoptotic cells waiting to be cleared is that they express PS on the outer surface of the membrane. Therefore, triggering antibody-dependent cellular phagocytosis (ADCP) with PS-specific antibodies can be considered as an important alternative for the treatment of such pathologies in which PS receptors are deficient. The possibility of such a specific monoclonal antibody already exists. Baviximab (PGN401) is a monoclonal human-mouse chimeric antibody directed against the membrane phospholipid PS. Baviximab and other antibodies that target PS were developed by Philip Thorpe's laboratory to specifically target tumor vasculature.^{15,104} Baviximab does not bind PS directly. It binds to PS-expressing membranes by crosslinking two molecules of β 2-glycoprotein 1 (β 2GPI1) bound to PS on the membrane.^{14,105} Baviximab could be expected to act by at least two mechanisms in severe COVID-19 patients: i) it can reduce the amount of virus to infect other tissues as a result of opsonization-mediated clearance of SARS-CoV2; ii) it induces ADCP of virus-infected apoptotic cells; thus, in pathologies where efferocytosis is defective, an opportunity arises for clearing apoptotic cells.¹⁰⁶

β 2GPI1 is an evolutionarily conserved single-chain anionic phospholipid-binding glycoprotein. It has gained much attention since the discovery of its role in antiphospholipid syndrome (APS).¹⁰⁷ Accordingly, β 2GPI1 binds to the PS expressed on apoptotic cells. When the amount of β 2GPI1 bound to PS reaches a certain threshold, antibodies dimerize the adjacent β 2GPI1 molecules. As a consequence, these high-affinity anti- β 2GPI1- β 2GPI1 complexes may activate targeted cells, causing anti-phospholipid antibodies-related manifestations.¹⁰⁸ However, during the clinical trials, baviximab therapy appeared safe and well tolerated, and reductions were observed in virus load in the blood. Furthermore, coagulation parameters remained within the normal range.^{106,109} In the light of those data and considering the FDA approval of the drug, I had planned a clinical trial to investigate the efficacy of baviximab treatment in patients with

severe COVID-19 in October 2020. Unfortunately, the opinion remained in theory, as OncXerna, the company that owns the drug, did not welcome this offer. Since the hallmark autoantibodies of APS are anti-beta2 glycoproteins, a series of fully human PS-targeting antibodies were generated by phage display technology.¹¹⁰ To further explore the antiviral activity of PS-targeting antibodies, my efforts to develop a specific antibody in my country, such as PGN632, that could bind directly to PS and would not need the cofactor β 2GP1 protein,¹⁰⁹ were also failed.

The majority of those who are infected with COVID-19 have a self-limiting infection and do recover. However, a minority with comorbidities proceeds to more severe disease, requiring intensive care unit admission.¹¹¹ Therefore, the disease may cease to be a threat following the treatment of patients who are in the risk group or whose condition deteriorates with the monoclonal anti-PS antibody to be developed. Moreover, such a treatment would also have the potential to control the morbidity and mortality caused by other enveloped viruses such as influenza, Ebola, dengue, and even HIV.

Vaccine and therapeutic development are predominantly focused on the essential virus-encoded Spike protein. However, the variation of the S gene between SARS-like CoV viruses and SARS-CoV-2 variants may affect the efficacy and particularly for cross-protection of a vaccine. Nowadays when mutations against vaccines are confusing, other proteins of SARS-CoV-2 seem to be important targets to develop alternative treatments against potential resistance or emerging new viruses in the future.¹¹² SARS-CoV-2 ORF3a is a putative viral ion channel implicated in autophagy inhibition, inflammasome activation, and apoptosis. As explained above, it is a specific viral protein that may be responsible for the development of the virus-induced apoptotic load and ultimately the adverse course of the disease in cases of defective efferocytosis.

SARS-CoV-2 enters the host cell by both membrane fusion and by clathrin/caveolin-mediated endocytosis after binding to the ACE2 cell-surface receptors.¹¹³ Caveolin-1 is a major component of caveolae membrane microdomains, which are involved in non-clathrin-mediated virus uptake into cells.¹¹⁴ Viral ORF3a protein binding to host caveolin-1 was shown to be essential for entry and endomembrane trafficking of SARS-CoV-2. In addition to viral entry, caveolin-1 was also associated with all stages of the viral life cycle.^{113,114} Even if ORF3a is expressed on viral particles, anti-ORF3a antibodies will not neutralize the virus by preventing attachment or fusion.¹¹⁵ In other words, antibodies that bind to ORF3a while disrupting the functions of the protein, the antibody-bound virus would be able to enter the cell. In turn, entry of the antibody-bound virus into the cell may present an opportunity for an unusual cytosolic antibody receptor, called TRIM21. Critically, TRIM21 is also a ubiquitin ligase and can efficiently target antibody-bound substrates for proteasomal degradation.¹¹⁶ Therefore, the development of an antibody or vaccine, targeting ORF3a, may provide more effective results in controlling severe cases of COVID-19 with a high risk of mortality.

Conclusion

Every virus infection and even mRNA vaccines have pro-apoptotic properties.¹¹⁷ Therefore, effective removal of this burden in cell death is inevitable for the maintenance of cellular homeostasis. Since defective efferocytosis is a potential threat to immune system dysregulation, a drug is urgently needed to support efferocytosis, “as it becomes more and more obvious that vaccination alone might not sufficient to curb the pandemic caused by this rapidly mutating virus”.¹¹⁸ Therefore, we will be awaiting further research in the hope that this work will be evaluated to respond to this urgent need.

Disclosure

The author reports no conflicts of interest in this work.

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