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HYPOTHESES

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S100A8 may govern hyper-inflammation in severe COVID-19

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic threatens human species with mortality rate of roughly 2%. We can hardly predict the time of herd immunity against and end of COVID-19 with or without success of vaccine. One way to overcome the situation is to define what delineates disease severity and serves as a molecular target. The most successful analogy is found in BCR-ABL in chronic myeloid leukemia, which is the golden biomarker, and simultaneously, the most effective molecular target. We hypothesize that S100 calcium-binding protein A8 (S100A8) is one such molecule. The underlying evidence includes accumulating clinical information that S100A8 is upregulated in severe forms of COVID-19, pathological similarities of the affected lungs between COVID-19 and S100A8-induced acute respiratory distress syndrome (ARDS) model, homeostatic inflammation theory in which S100A8 is an endogenous ligand for endotoxin sensor Toll-like receptor 4/Myeloid differentiation protein-2 (TLR4/MD-2) and mediates hyper-inflammation even after elimination of endotoxin-producing extrinsic pathogens, analogous findings between COVID-19-associated ARDS and pre-metastatic lungs such as S100A8 upregulation, pulmonary recruitment of myeloid cells, increased vascular permeability, and activation coagulation cascade. A successful treatment in an animal COVID-19 model is given with a reagent capable of abrogating interaction between S100A8/S100A9 and TLR4. In this paper, we try to verify our hypothesis that S100A8 governs COVID-19-associated ARDS.

KEYWORDS

acute respiratory distress syndrome, Coronavirus disease 2019, myeloid-derived suppressor cells, S100A8, Toll-like receptor

Abbreviations: BCR-ABL, breakpoint cluster region-Abelson murine leukemia; CCL2, chemokine (C-C motif) ligand 2; CXCL1, C-X-C Motif Chemokine Ligand 1; CXCL11, C-X-C Motif Chemokine Ligand 11; EBvirus, Epstein-Barr virus; FOXJ1, forkhead box J1; HFH4, hepatocyte nuclear factor-3/forkhead homologue 4; HMGB1, high-mobility-group box protein 1; IL-6, interleukin-6; RAGE, receptor for advanced glycosylation end; RIG-I, retinoic-acid inducible gene I; SAA3, serum amyloid A3; TLR, Toll-like receptor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

1 | PREFACE

The pandemic allows severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to acquire mutations after tremendous chances of replication in human body. The mutations potentially provide the virus with abilities to enhance infectivity through altered adhesion machinery, escape immune protection and vaccination, and spread beyond species, as evidence by Indian strains.[1–3] Artificial mutation of the virus can even cause infection to mice.[4] Given that we can hardly predict the end of the infected world, it can safely be said that we definitely need to struggle against life-threatening severe manifestations of COVID-19. The common but often lethal complication of COVID-19 is acute respiratory distress syndrome (ARDS)[5] as observed in 81% of the 52 critically ill patients in 710 cases with SARS-CoV-2 pneumonia.[6] Without metastasis, we can survive cancer. Without the severity, we can survive the pandemic without fear.

The hypothesis should originate from the most reliable lung pathology of severe cases of COVID-19, and be established by the results from logically designed animal experiments. Since COVID-19 is a systemic infection initiated in respiratory tracts, the hypothesis is given from the standpoint of whole body but supported by molecular and cellular levels of experimental evidence.

2 | DEFINITION OF TERMS

We understand that "hypothesis" (hereinafter, we put double quotation marks for the terms that we need to define) means a proposed idea based on a limited but significantly evaluated set of evidence at the time of presentation, needs to be testable in a repeated fashion, and eventually raises a perspective for further accumulation of data to reach the level of theory and truth at least acceptable in the scientific community. Therefore, we tentatively explain the ARDS associated with COVID-19 with our hypothesis. Pathogens including bacteria and virus and their components such as endotoxin are basically of exogenous origin. A receptor inherent in mammals capable of sensing endotoxins can also serve as a receptor for molecules of endogenous origin. Those molecules are called "endogenous ligands" as represented by S100A8 and HMGB1 binding to TLR4 that has been believed to be a pathogen sensor recognizing endotoxins of Gram-negative rods. S100A8 expression is upregulated in pre-metastatic lungs. "Pre-metastasis" is a concept by which to explain the tissue microenvironment without tumor cells before the actual arrival of metastasizing tumor cells from other organ as a primary site.[7] "Inflammation" is defined to manifest two essential features, that is, leukocyte mobilization and increased vascular permeability. Defending against constant assaults

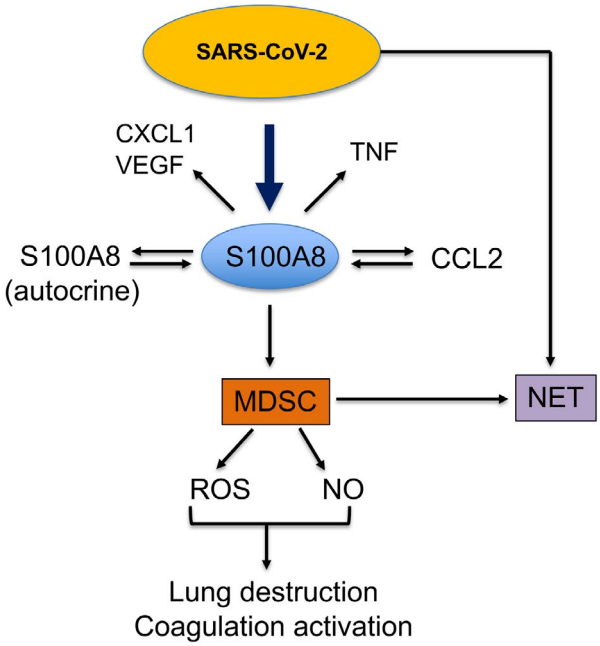

FIGURE 1 S100A8 is the headquarters. SARS-CoV-2 induces upregulation of S100A8 possibly through pattern recognition receptors such as TLR. S100A8 not only amplifies itself but also induces a set of key cytokines/chemokines including CCL2, VEGF, CXCL1, and TNF, resulting in recruitment of immune-suppressive myeloid cells (MDSCs) in the lungs. MDSCs work in concert with platelets to provoke NET. Activation of coagulation is enhanced by irreversible tissue injury triggered by production of two-atom molecules such as reactive oxygen species (ROS) and nitrogen oxide (NO). Inhibition of S100A8 may be the most effective therapy to stop lung destruction during the reversible stage

of air-borne pathogens as a danger, pulmonary recruitment of leukocytes and their extravasation take place even in healthy individuals in a physiological manner. In accordance with danger hypothesis proposed by Matzinger,[8] we have proposed to call this low level of inflammation as "homeostatic inflammation".[9–13] In response to exogenous stimuli, the levels of inflammation could rise to be called "hyper-inflammation" and sustained. The so-called cytokine storm meaning elevated expression of cytokines and chemokines causes hyper-inflammation.

3 | EVIDENCE FROM PATIENTS

Plasma levels of S100A8 and CXCL11 are significantly elevated in severe cases[14] (Figure 1). Therapy against less significantly elevated IL-6 failed to decrease the mortality of severe COVID-19 pneumonia at 28 days.[15] Irrespective of superimposed bacterial infection in severe cases, S100A8 is released and its plasma concentration correlated with counts of immature form of neutrophils, and intriguingly with levels of two coagulation factors, fibrinogen and D dimer.[14]

Interestingly, those immature neutrophils are $CD10^{low}CD101^{-}CXCR4^{+/-}$ whose transcription profile revealed upregulation of genes in the reactive oxygen species (ROS) production and nitric oxygen species (NOS), implicating their property as myeloid-derived suppressor cells (MDSC)[16] (Figure 1). Since human MDSC biomarkers are not well defined, functional markers including potential production of ROS and NOS can give the reliable phenotype.[17] Cell components in bronchoalveolar lavage fluid (BALF) revealed a prominent increase in neutrophils in COVID-19 as compared with other pneumonias.[18] This raises at least four fundamental questions of, [1] which cells specifically produce S100A8 upon infection, [2] whether or not S100A8 upregulation is specific to SARS-CoV-2, [3] why canonical antibacterial neutrophils transmigrate through epithelial barrier to alveolar space in this particular viral infection, and [4] what connects infection with activation of coagulation cascade.

3.1 | Questions 1 and 2

In primary cultures of human bronchial epithelial cells, the infection takes place initially in ciliated cells in 84% and then spread to other types of cells.[19] A comparative transcript analysis between SARS-CoV-2 and other airway viruses such as influenza H1N1 and SARS revealed that S100A8 is specifically upregulated in SARS-CoV-2.[20]

3.2 | Question 3

Intra-tracheal infection of SARS-CoV-2 to rhesus macaque monkeys provoked increased neutrophil counts as well as their markers such as myeloperoxidase and expression of S100A8 in the affected lungs at 3 and 5 days post-infection (dpi).[21] The expression profile showed upregulation of cellular response genes to endotoxin or lipopolysaccharide (LPS) responsible for neutrophil chemotaxis, while type I interferon was not induced. This is understandable since the upregulated S100A8 is an endogenous ligand of TLR4, namely an endogenous equivalent to LPS (see below). Given that our pulmonary pre-metastasis theory based on the S100A8-SAA3 axis[10,12,13] was flawed and could not be applied to human due to one base insertion mutation in the SAA3 gene in more evolved species chimpanzee and human resulting in inactivation of SAA3 as pseudo-gene, we should always be careful to interpret non-human animal results.

3.3 | Question 4

The pathological findings of ARDS in COVID-19 patients from different researchers also revealed inflammation with coagulopathy and cell death including diffuse alveolar damage, hyaline membrane formation, interstitial edema, infiltrating lymphocytes, and microvascular thromboemboli.[22] They also documented viremia in those autopsy samples and this is followed by other similar reports.[23] However, transmission of SARS-CoV-2 by transfusion of blood from asymptomatic donors is rare.[24] Japanese Red Cross Society does not currently perform detection of SARS-CoV-2 in donated blood.[25]

Post-mortem analysis of COVID-19 patients, that is, patients accompanied by severe tissue injury and release of the virus, revealed inflammatory involvement of endothelial cells such as apoptosis.[26] The detrimental effects on the homeostatic endothelial barrier result in activation of coagulation. It is no wonder given that SARS-CoV-2 receptor is angiotensin-converting enzyme 2 (ACE2) expressed not only in respiratory epithelial cells but also endothelial cells. First, this may allow the virus to get access to circulation. Second, involvement of exosome is also implicated as in the case of cancer patients.[27,28] We should bear in mind that the idea of exosome originates from discovery of micro-vesicles released from Hodgkin cells from an EB-virus-infected patient.[29] Therefore, in severe cases as accompanied by ARDS whose blood is not usually used for transfusion, it is no wonder that the virus circulates in blood stream.

4 | EVIDENCE FROM ANIMAL MODELS

To accumulate detailed pathological evidence with time-course changes, we need mouse models of COVID-19. However, we should be cautious about the differences and similarities between human and mouse in apparent features of non-lymphocytic white blood cells (WBC) involved in innate immunity. In human, 50%-70% of circulating WBC are neutrophils, whereas only 10%-25% in mice with no gender difference when collected from tail vein.[30] Labeling experiments in human volunteers showed that circulating neutrophils accounted for roughly 70% of total with a mean half-life (t1/2) being 10.4 hours and 30% were marginalized from circulation.[31] The t1/2 of $Gr\text{-}1^{+}$ neutrophils in mouse experiments showed 11.4 hours in the wild-type mice.[32,33] Gr-1 is expressed in immature myeloid cells and its expression level increases along with cell maturation. The anti-Gr-1 antibody RB6-8C5 dually reacts with Ly-6G and Ly-6C, both of which are expressed in murine neutrophils. Thus, almost no difference in survival time was observed between human and mouse.

Due to interspecies difference between human and mouse in the ability of viral spike protein as adhesion machinery to bind host ACE2, SARS-CoV-2 can hardly make an entry into murine epithelial cells. To facilitate virus entry into murine lung epithelial cells to recapitulate COVID-19 pneumonia,

cytokeratin-18 promoter-driven transgenic (tg) mice of human ACE2 (hACE2) were established.[34] The detailed findings in the intra-nasally infected mice include focal existence of viral RNAs in alveolar epithelial cells and Ly-6G$^+$ neutrophils and Ly-6C$^+$ monocytes in the perivascular regions at 2 dpi, extension of those leukocytes to near alveolar spaces at 4 dpi and diffusely localized viral RNAs, elevated D dimer at 2 and 4 dpi, large spread of those cells in alveolar space and edematous interstitium and prolonged prothrombin time at 7 dpi. Of note, levels of viral RNAs, which co-localized with dead cell debris but not with immune cells alive, decreased by 7 dpi as examined by in situ hybridization. The virus can hardly replicate in dead cells. S100A8 was upregulated in the lungs at 5 dpi, which was not observed after influenza A virus infection of the same recombinant mice.[21] Another hACE2 tg mice with ciliated cell-specific HFH4/FOXJ1 promoter exhibited similar pathological findings after infection with mortality rate of 50%, including hyaline thrombus and fibrin in mice with severe pneumonia.[35] S100A8 binds TLR4 and RAGE, inhibition of which by Paquinimod and Azeliragon, respectively, turned out to be effective only in the abrogation of the S100A8-TLR4 binding.[21]

5 | HYPOTHESIS

Lung metastasis and COVID-19 pneumonia share many points including (1) S100A8 as a key molecule, (2) pulmonary inflammation with MDSC mobilization from bone marrow and increased vaso-permeability, and (3) an idea of conversion of stimuli from extrinsic to intrinsic.

1. S100A8 as a key molecule

S100A8 is an EF-hand Ca^{2+}-binding protein, which can hetero-dimerize with its highly related protein S100A9, and is an early response chemokine in innate immunity to danger such as cancer and infection. Abundantly expressed in neutrophils, S100A8 and S100A9 constitute approximately 45% of cytosolic proteins of neutrophils.[36]

S100A8 was known long as L1 antigen of granulocytes[37] before the biological connections were proposed, including rheumatoid arthritis[38] and cystic fibrosis (CF).[39] CF is an autosomal recessive multiple-organ disease with mutations in the CF transmembrane conductance regulator (CFCR) gene responsible for chloride channel in epithelial cells. One of the prominent features is chronic inflammation with elevated S100A8 expression in the lungs. Importantly, elevated S100A8 expression is lung specific and not observed in liver and ileum. CFCR-disrupted C57BL/6 mice manifest pneumonia, which interestingly takes place in a sterile manner, that is, even before bacterial infection.[40] This is followed by a vicious cycle of inflammation and infection due to defect in bacterial clearance caused by S100A8-mobilized neutrophils injurious to the lungs by producing reactive oxygen species (ROS). A recent study shows an involvement of platelet activation in the aggravation of pneumonia after intra-tracheal LPS challenge.[41] It is known that neutrophils collaborate with platelets to trap bacteria, which is called neutrophilic extracellular trap (NET) (Figure 1). NET depends on TLR4 on the platelets.[42] However, SARS-CoV-2 directly induces NET in neutrophils, which causes epithelial cell death at least in vitro.[43]

We have shown that S100A8 expression is upregulated in endothelial cells and macrophages in pre-metastatic lungs as well as in the serum by the primary tumor-derived secretome including CCL2 and exosomes.[7,9,44,45] Coagulation activation was observed in pre-metastasic lung as represented by the presence of fibrin/fibrinogen deposits.[45] We and other group have demonstrated that S100A8 serves as an endogenous ligand for Toll-like receptor 4/Myeloid differentiation protein-2 (TLR4/MD-2) complex whose authentic ligand is endotoxin of lipopolysaccharide (LPS) of exogenous origin.[44,46–48] Given that S100A8 enhanced the formation of pre-metastatic lung microenvironment and that antibody-mediated inhibition of S100A8 and eritoran, an inhibitor against TLR4/MD-2 complex, could abrogate lung metastasis and pre-metastatic niche formation, respectively, the S100A8-TLR4/MD-2 axis is necessary and sufficient for pre-metastatic niche establishment and subsequent lung metastasis.

Collectively, pulmonary S100A8 expression is augmented in COVID-19 pneumonia, CF lungs, and pre-metastatic lungs, and is supposed to be responsible for each of the pathogenesis as commonly mediated by TLR4. Massive recruitment of neutrophils destroys lung tissues. S100A8-dependent pulmonary recruitment of MDSC is required for pre-metastatic niche formation in the lungs. Given that S100A8 expression in the lungs is augmented in a variety of cancers in our hands,[45] we predict that tumor burden should be able to aggravate COVID-19 pneumonia. In fact, an epidemiological report from Wuhan showed that frequency of severe pneumonia was 32% vs 64% in statistically matched COVID-19 patients without and with cancer.[49] Significantly more elevated TNF and IL-6 were detected in cancer patients.

2. MDSC recruitment and increased vaso-permeability in the lungs

Pre-metastasis is an inflammatory phenomenon that takes place in a sterile fashion. Two essential features are leukocyte recruitment and increased vascular permeability. Leukocyte mobilization to the lungs from bone marrow and subsequent extravasation are observed in both pre-metastatic lung and COVID-19 pneumonia. Both LPS and S100A8 regulate expression of CD11b, an integrin responsible for neutrophil accumulation in the lungs.[50,51] Increased numbers of

$CD11b^{+}Gr\text{-}1^{+}$ myeloid cells (equivalent to MDSC) are observed in pre-metastatic lungs in murine models and in fact they constitute pre-metastatic niche.[7]

In a murine ARDS model with LPS, and intriguingly also with S100A8, we observed a similar pattern of cell recruitment (Figure 2A). Forty-eight hr after intratracheal administration of LPS or S100A8, inflammatory responses as indicated by infiltrating leukocytes and lymphocytes, and swelling of alveolar epithelial cells were sparsely observed (Figure 2A), which are pathological changes mainly observed in COVID-19-mediated ARDS.[52] In addition, hyaline membrane, which is supposed to be exudative products formed by increased permeability of capillary vessels, was observed in alveolar space of SARS-CoV-2-induced pneumonia patients.[52] However, in the case of mouse models, hyaline membrane could be hardly detected in ALI (acute lung injury)/ARDS model mice.[53] Recently, Hong et al showed that SARS-CoV-2 infection induced hyaline membrane-like changes in hACE2-transgenic mice.[54] In our model, at 5 days after the treatment, similar to Hong's findings, the so-called hyaline membrane-like changes were present in S100A8- or LPS-treated mice (Figure 2A). Reizine et al recently reported that immunosuppressive M-MDSCs and PMN-MDSCs were accumulated in patients with severe COVID-19.[55] As mentioned above, murine $CD11b^{+}Gr\text{-}1^{+}$ cells can be divided into two groups, that is, $CD11b^{+}Ly6C^{low}Ly6G^{+}$; polymorphonuclear-MDSCs (PMN-MDSCs) and $CD11b^{+}Ly6C^{high}Ly6G^{-}$; monocytic-MDSC (M-MDSCs) populations. The pulmonary recruitment of PMN-MDSCs and M-MDSCs was significantly elevated in intratracheally treated mice with S100A8 or LPS (Figure 2B). Moreover, both M-MDSCs and PMN-MDSCs are increased in the peripheral blood of S100A8 or LPS-treated mice (Figure 2C). Since S100A8 was significantly elevated in the serum of LPS-treated mice, these results suggested that S100A8 would play a crucial role also in ARDS. While the contrast enhancement effect indicating increased vascular permeability is the reliable

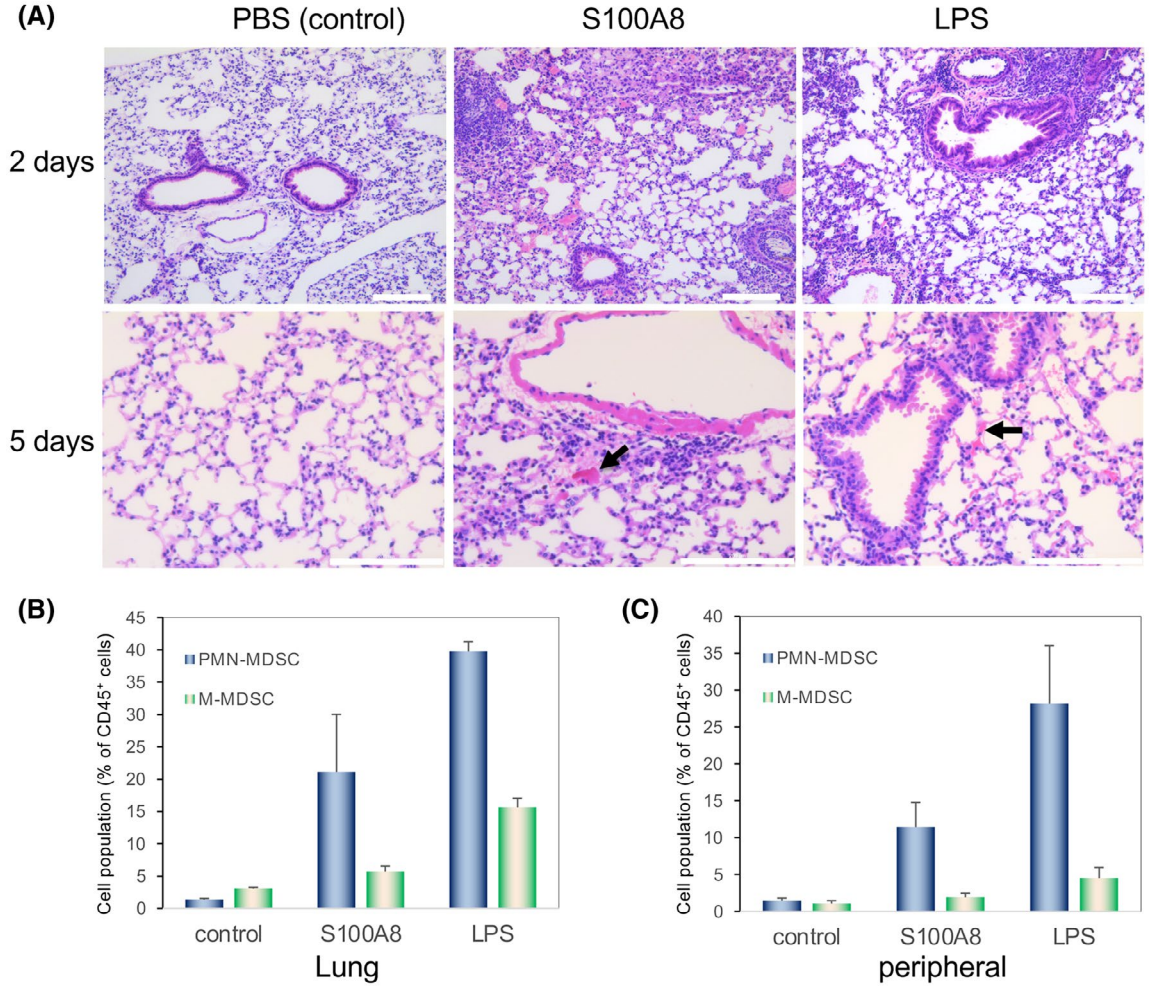

FIGURE 2 S100A8 induces ARDS-like pathology. A, Pulmonary pathological changes in mice treated with S100A8 or LPS. Arrows indicate hyaline membrane-like changes (see text for details) in alveolar cavities. Scale bars represent 200 μm. B, LPS induces pulmonary recruitment of PMN-MDSCs and M-MDSCs in mice. S100A8, to lesser content, induces pulmonary recruitment of these two populations. C, In addition to LPS, the intratracheal administration of S100A8 increases cell numbers of PMN-MDSCs and M-MDSCs in peripheral blood

indicator in CT-mediated diagnosis of pneumonia in clinics, animal experiments with enhanced Evans blue leakage also represents pre-metastatic niche foci with high expression of S100A8 and CCR2 (CCL2 receptor).[45] In contrast to severe pneumonia in COVID-19 in which high levels of inflammation destroys gas-exchange alveolar machinery, it is likely that pre-metastatic lungs still retain blood oxygen levels. However, gas-exchange is strongly impaired in lymphangitis carcinomatosa, the most severe case of lung metastasis.[56]

The pathological findings of ARDS in COVID-19 revealed inflammation with cell death and coagulopathy including diffuse alveolar damage, hyaline membrane formation, interstitial edema, infiltrating lymphocytes, and microvascular thromboemboli.[22] SARS-CoV-2 is positive single-stranded RNA virus, and can be recognized by pattern recognition receptors (PRRs) including TLR7, TLR3, and RIG-I capable of inducing apoptosis and subsequent elimination of infected cells.[57,58] Although alveolar damages are certainly observed in the foci of lung metastasis due to tumor cell invasion but not observed in pre-metastatic lungs that lack tumor cells, activation of coagulation cascade is manifested as fibrinogen/fibrin deposition in pre-metastatic lungs.[45] As shown in Figure 3, the lungs are characterized by the interphase between air and circulation. LPS derived from bacteria, viral RNAs of SARS-CoV-2 are recognized by TLR4 and TLR7, respectively, in the epithelial cells, which leads to the induction of a subset of cytokines/chemokines through the paracrine cascade mediated by interstitial cells and endothelial cells, resulting in serum elevation of S100A8. S100A8 stimulates mobilization of TLR4-expressing leukocytes such as MDSCs from bone marrow (bone marrow-derived myeloid cells: BMDC) into the lungs, which extravasate into the interstitial and alveolar space. In pre-metastasis, primary-tumor-derived secretome including CCL2 induces expression of S100A8 in the lung endothelial and interstitial cells such as macrophages, which, in turn, stimulates mobilization of leukocyte from bone marrow via circulation. BMDC serves as the essential component of pre-metastatic niche that accommodates metastasizing tumor cells.

3. An idea of conversion of stimuli from extrinsic to intrinsic

Although LPS or viral RNAs levels decrease over time in the infected tissues, endogenous mediators induced by them can make a vicious cycle for production in the surrounding cells or even distant cells in a paracrine and endocrine manner, respectively. Both LPS and SARS-CoV-2 can induce expression of S100A8, which, in turn, can auto-amplify its own gene expression in a manner dependent on TLR4[21] (Figure 1). Therefore, even after elimination of microbes or viruses by antibiotics, antibodies or whatever, if levels of vicious cycle for auto-amplification are high enough to result in systemic inflammation with the so-called cytokine storm, lethal consequences may occur. Cytokine storm is an ambiguous word and never means any particular set, order, strength, specific producer, and responder cells of cytokines and chemokines in an organized manner. This means that initial extrinsic pathogen-derived stimuli as a trigger that are sensed by TLRs can make a conversion to intrinsic mediators that can also directly signal through TLRs, and that host misunderstands that pathogens are persistent in the infected tissue and mobilizes MDSC from bone marrow. Therefore, it can be said that augmented expression of endogenous ligand of TLR4 is the deterioration of homeostatic inflammation. Most of the cytokines are chemokines at the same time capable inducing cell mobilization and vascular permeability, and therefore the levels of inflammation are expected to be high and could be irreversible. In addition to S100A8 by itself, S100A8 is capable of inducing significant expression of TNF, CXCL1, CCL2, and VEGF in a variety of cells including endothelial cells, epithelial cells, and myeloid cells.[62] In human cholangiocarcinoma cells, S100A8 can upregulate VEGF in a TLR4-dependent manner to facilitate their liver metastasis in a murine model.[63] Established pre-metastatic milieu could be reversible upon removal of transplanted tumor in animal models.[7] Detection and inhibition of S100A8 at early stage of severe COVID-19 might provide efficient treatments before the disease enters an irreversible process.

6 | PROPOSAL FOR DRUG DISCOVERY

If the COVID-19 ARDS is irreversible and levels of injured lungs are high enough to impair gas-exchange, it is fatal. However, if we detect elevation of S100A8 in the serum and BALF at the early stages and inhibitors against the S100A8-TLR4 system are administered to the patients, we may cure the disease.

We would like to repeatedly underline that the pre-metastatic process in the lungs is reversible at least in our hands.[7]

Lessons learned from clinical trials are useful. The first drug proven to diminish deaths in patients with severe lung complications, but not with no respiratory support, is 10 days dexamethasone, a well-known anti-inflammatory steroid,[64,65] although corticosteroids in general have not been routinely recommended in ARDS therapy.[66]

Very recently, Eisai launched a clinical trial of eritoran against moderate to severe cases of COVID-19, an LPS analog antagonizing LPS via TLR4/MD-2.[67] The

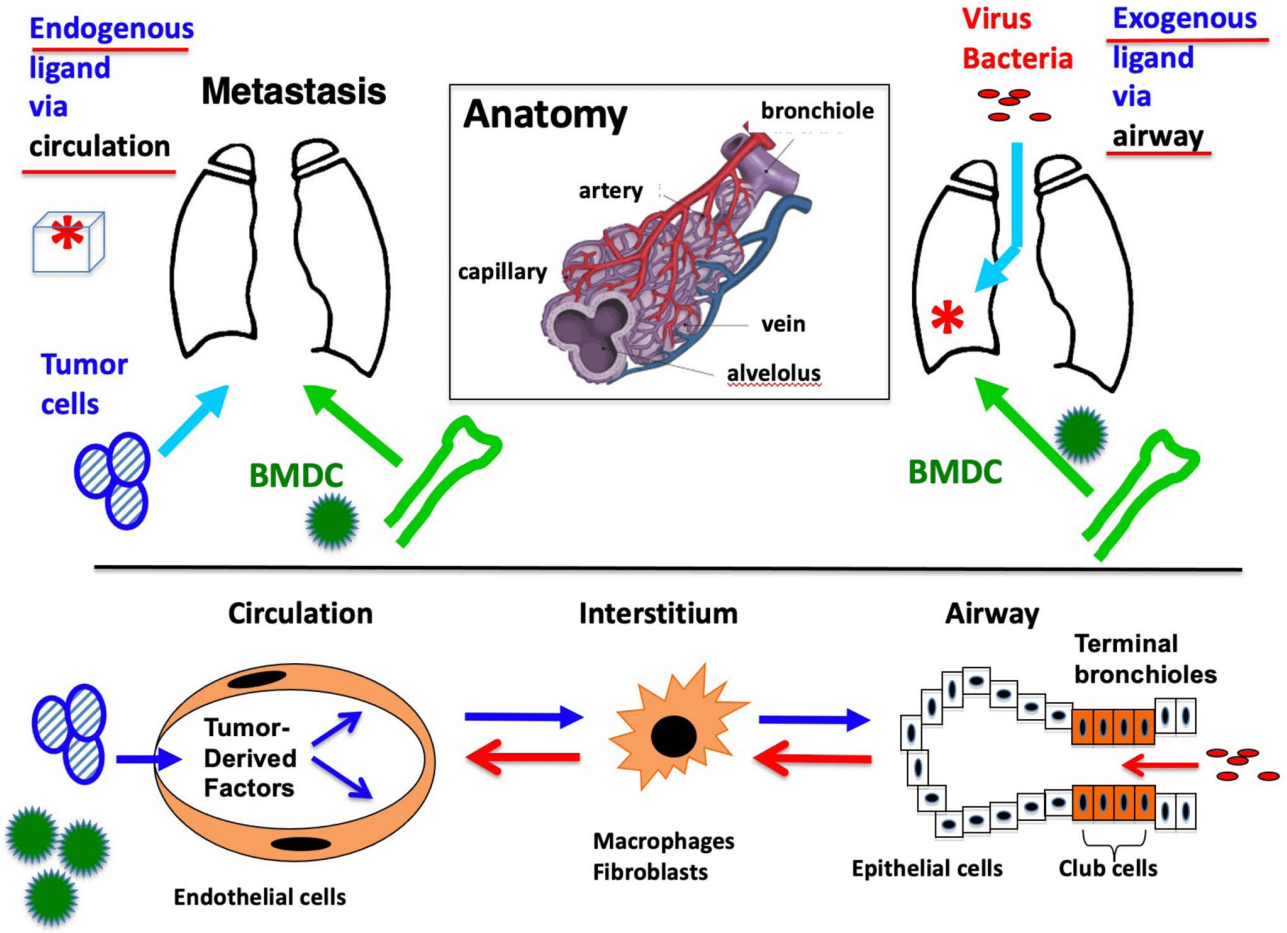

FIGURE 3 Paracrine cascades in the lungs (modified from Refs. 12,13). Triggering molecular events such as sensing RNA viruses in the airway epithelial cells require direct or indirect (via interstitial and/or endothelial cells) transfer of signals to circulation, which, in turn, mobilizes white blood cells (including MDSC shown in Figure 1) from bone marrow in an endocrine manner. Recruited cells penetrate through pulmonary endothelial cells into interstitial and alveolar space. However, the triggering events in COVID-19 are big enough to induce trans-epithelial migration of myeloid cells as evidenced in clinical settings. Anti-S100A8 antibody was shown to block anti-epithelial migration of leukocytes induced by endotoxin[59–61]

former trial of the same drug against septic shock failed in Phase III in 2014. Although severe COVID-19 patients at end stages might have sepsis, eritoran was selected as an immuno-modulator. What do you think is the insight here? We showed evidence that eritoran can abrogate establishment of the pulmonary pre-metastatic microenvironment that facilitates tumor cell entry in the lungs.[48] We suppose that the true facilitator for lung metastasis is S100A8, not LPS, and eritoran may compete with S100A8 for binding TLR4/MD-2.[48] Our hypothesis makes us wish discoveries of effective drugs against the S100A8-TLR4/MD-2 system.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

A. Deguchi and Y. Maru designed the research; A. Deguchi, T. Yamamoto, and N. Shibata performed the research; A. Deguchi and Y. Maru wrote the paper. All authors have read and approved the final submitted manuscript.

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