HYPOTHESES

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; HPH4, 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.

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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 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 CD10lowCD101−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).

Cell components in bronchoalveolar lavage fluid (BALF) revealed a prominent increase in neutrophils in COVID-19 as compared with other pneumonias. 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 antibiotic 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. 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.

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). 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 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. They also documented viremia in those autopsy samples and this is followed by other similar reports. However, transmission of SARS-CoV-2 by transfusion of blood from asymptomatic donors is rare. Japanese Red Cross Society does not currently perform detection of SARS-CoV-2 in donated blood.

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. 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. 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. 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. 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. The t1/2 of Gr-1+ neutrophils in mouse experiments showed 11.4 hours in the wild-type mice. 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. 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. 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. 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.

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.

S100A8 was known long as L1 antigen of granulocytes before the biological connections were proposed, including rheumatoid arthritis and cystic fibrosis (CF). CF is an autosomal recessive multiple-organ disease with mutations in the CF transmembrane conductance regulator (CFTR) 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. CFTR-disrupted C57BL/6 mice manifest pneumonia, which interestingly takes place in a sterile manner, that is, even before bacterial infection. 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. 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. However, SARS-CoV-2 directly induces NET in neutrophils, which causes epithelial cell death at least in vitro.

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. Coagulation activation was observed in pre-metastatic lung as represented by the presence of fibrin/fibrinogen deposits. 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. 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, 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. 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. Increased numbers of
CD11b+Gr-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-1+ cells can be divided into two groups, that is, CD11b+Ly6C<sub>low</sub>Ly6G<sup>+</sup>; polymorphonuclear-MDSCs (PMN-MDSCs) and CD11b+Ly6C<sub>high</sub>Ly6G<sup>-</sup>; 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
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). 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.

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. 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. 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. 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 (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 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. In human cholangiocarcinoma cells, S100A8 can upregulate VEGF in a TLR4-dependent manner to facilitate their liver metastasis in a murine model. Established pre-metastatic milieu could be reversible upon removal of transplanted tumor in animal models. 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.

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, although corticosteroids in general have not been routinely recommended in ARDS therapy. 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. The
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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REFERENCES
1. Rodriguez RA, Pepper IL, Gerba CP. Application of PCR-based methods to assess the infectivity of enteric viruses in environmental samples. Appl Environ Microbiol. 2009;75:297-307.
2. Hodgson SH, Mansatta K, Mallett G, Harris V, Emary KRW, Pollard AJ. What defines an efficacious COVID-19 vaccine? A
review of the challenges assessing the clinical efficacy of vaccines against SARS-CoV-2. *Lancet Infect Dis*. 2021;21:e26-e35.

3. Dash P, Turuk J, Behera SK, et al. Sequence analysis of Indian SARS-CoV-2 isolates shows a stronger interaction of mutant receptor-binding domain with ACE2. *Int J Infect Dis*. 2021;104:491-500.

4. Dinnon KH 3rd, Leist SR, Schäfer A, et al. A mouse-adapted model of SARS-CoV-2 to test COVID-19 countermeasures [published correction appears in Nature. 2021 Feb; 590(7844):E22]. *Nature*. 2020;586:560-566.

5. Rawal G, Yadav S, Kumar R. Acute respiratory distress syndrome: an update and review. *J Transl Int Med*. 2018;6:74-77.

6. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med*. 2020;8:475-481.

7. Maru Y. Premetastasis. *Cold Spring Harbor Perspect Med*. 2020;10:a036897.

8. Matzinger P. The danger model: a renewed sense of self. *Science*. 2002;296:301-305.

9. Maru Y. A concept of homeostatic inflammation provided by endogenous TLR4 agonists that function before and after danger signal for metastasis. *Antiinflamm Antiallergy Agents Med Chem*. 2009;8:337-347.

10. Tomita T, Sakurai Y, Ishibashi S, Maru Y. Imbalance of Clara cell-mediated homeostatic inflammation is involved in lung metastasis. *Oncogene*. 2011;30:3429-3439.

11. Miyake K, Kaisho T. Homeostatic inflammation in innate immunity. *Curr Opin Immunol*. 2014;30:85-90.

12. Maru Y. *Inflammation and Metastasis*. 1st ed. Springer; Tokyo, 2016.

13. Maru Y. *Inflammation and Metastasis*. 2nd ed. Springer; Tokyo, 2021 in press.

14. Silvin A, Chapuis N, Dunsmore G, et al. Elevated calprotectin and abnormal myeloid cell subsets discriminate severe from mild COVID-19. *Cell*. 2020;182:1401-1418.e18.

15. Rosas IO, Brutu N, Waters M, et al. Tocilizumab in hospitalized patients with severe Covid-19 pneumonia. *N Engl J Med*. 2021;384:1503-1516.

16. Veglia F, Perego M, Gabriovich D. Myeloid-derived suppressor cells coming of age. *Nat Immunol*. 2018;19:108-119.

17. Bruger AM, Dorhoi A, Esendagli G, et al. How to measure the immunosuppressive activity of MDSC: assays, problems and potential solutions. *Cancer Immunol Immunother*. 2019;68:631-644.

18. Zhou Z, Ren L, Zhang L, et al. Heightened innate immune responses in the respiratory tract of COVID-19 patients. *Cell Host Microbe*. 2020;27:883-890.e2.

19. Ravindra NG, Alfajaro MM, Gasque V, et al. Single-cell longitudinal analysis of SARS-CoV-2 infection in human airway epithelium identifies target cells, alterations in gene expression, and cell state changes. *PLoS Biol*. 2021;19:e3001143.

20. Chandrashekar DS, Manne U, Varambally S. Comparative transcriptome analyses reveal genes associated with SARS-CoV-2 infection of human lung epithelial cells. *bioRxiv*. 2020. https://doi.org/10.1101/2020.06.24.169268

21. Guo Q, Zhao Y, Li J, et al. Induction of alarmin S100A8/A9 mediates activation of aberrant neutrophils in the pathogenesis of COVID-19. *Cell Host Microbe*. 2021;29:222-235.e4.

22. Wichmann D, Spahrke J-P, Lüthgeheymann M, et al. Autopsy findings and venous thromboembolism in patients with COVID-19: a prospective cohort study. *Ann Intern Med*. 2020;173:268-277.

23. Li Y, Schneider AM, Mehta A, et al. SARS-CoV-2 viremia is associated with distinct proteomic pathways and predicts COVID-19 outcomes. *medRxiv*. 2021. https://doi.org/10.1101/2021.02.24.21252357

24. Cappy P, Candotti D, Sauvage V, et al. No evidence of SARS-CoV-2 transfusion transmission despite RNA detection in blood donors showing symptoms after donation. *Blood*. 2020;136:1888-1891.

25. http://www.jrc.or.jp/jr/news/pdf/yuketsuj-2004COVID-19.pdf

26. Varga Z, Flammer AJ, Steiger P, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet*. 2020;395:1417-1418.

27. Elrasheid F, Aljaddawi AA, Redwan EM, Eversky VN. On the potential role of exosomes in the COVID-19 re-infection/reactivation opportunity [published online ahead of print, 2020 Jul 9]. *J Biomol Struct Dyn*. 2020;1-12.

28. Whiteside TL. The emerging role of plasma exosomes in diagnosis, prognosis and therapies of patients with cancer. *Contemp Oncol*. 2018;22:38-40.

29. Friend C, Marovitz W, Henie G, et al. Observations on cell lines derived from a patient with Hodgkin’s disease. *Cancer Res*. 1978;38:2581-2591.

30. Deding DC, Borowicz JL, Crockett ET. Gender dimorphism in differential peripheral blood leukocyte counts in mice using cardiac, tail, foot, and saphenous vein puncture methods. *BMC Clin Pathol*. 2003;3:3.

31. Price TH, Chatta GS, Dale DC. Effect of recombinant granulocyte colony-stimulating factor on neutrophil kinetics in normal young and elderly humans. *Blood*. 1996;88:335-340.

32. Lee PY, Wang JX, Parisini E, Dascher CC, Nigrovic PA. Ly6 family proteins in neutrophil biology. *J Leukoc Biol*. 2013;94:585-594.

33. Basu S, Hodgson G, Katz M, Dunn AR. Evaluation of role of G-CSF in the production, survival, and release of neutrophils from bone marrow into circulation. *Blood*. 2002;100(3):854-861.

34. Winkler ES, Bailey AL, Kafai NM, et al. SARS-CoV-2 infection of human ACE2-transgenic mice causes severe lung inflammation and impaired function. *Nat Immunol*. 2020;21:1327-1335.

35. Jiang R-D, Liu M-Q, Chen Y, et al. Pathogenesis of SARS-CoV-2 in transgenic mice expressing human angiotensin-converting enzyme 2. *Cell*. 2020;182:50-58.e8.

36. Edgeworth J, Gorman M, Bennett R, Freemont P, Hogg N. Identification of p8,14 as a highly abundant heterodimeric calcium binding protein complex of myeloid cells. *J Biol Chem*. 1991;266:7706-7713.

37. Ortiz-Muñoz G, Yu MA, Lefrançais E, et al. Cystic fibrosis transmembrane conductance regulator dysfunction in platelets drives lung hyperinflammation. *J Clin Invest*. 2020;130:2041-2053.
42. Clark SR, Ma AC, Tavener SA, et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat Med*. 2007;13:463-469.

43. Veras FP, Pontelli MC, Silva CM, et al. SARS-CoV-2-triggered neutrophil extracellular traps mediate COVID-19 pathology. *J Exp Med*. 2020;217:e20201129.

44. Hiratsuka S, Watanabe A, Aburatani H, Maru Y. Tumour-mediated upregulation of chemotactic proteins and recruitment of myeloid cells determines lung metastasis. *Nat Cell Biol*. 2006;8:1369-1375.

45. Hiratsuka S, Ishibashi S, Tomita T, et al. Primary tumours modulate innate immune signalling to create pre-metastatic vascular hyperpermeability foci. *Nat Commun*. 2013;4:1853.

46. Vogl T, Tenbrock K, Ludwig S, et al. Mrp8 and Mrp14 are endogenous activators of Toll-like receptor 4, promoting lethal, endotoxin-induced shock. *Nat Med*. 2007;13:1042-1049. https://doi.org/10.1038/nm1638

47. Deguchi A, Tomita T, Ohto U, et al. Eritoran inhibits S100A8-serum amyloid A3-TLR4 paracrine cascade establishes a pre-metastatic phase. *Nat Cell Biol*. 2008;10:1349-1355.

48. Deguchi A, Tomita T, Ohno U, et al. Eritoran inhibits S100A8-mediated TLR4/MyD-2 activation and tumor growth by changing the immune microenvironment. *Oncogene*. 2016;35:1445-1456.

49. Tian J, Yuan X, Xiao J, et al. Clinical characteristics and risk factors associated with COVID-19 disease severity in patients with cancer in Wuhan, China: a multicentre, retrospective, cohort study. *Lancet Oncol*. 2020;21:893-903.

50. Yipp BG, Kim JH, Lim R, et al. The Lung is a Host Defense Niche for Immediate Neutrophil-Mediated Vascular Protection. *Sci Immunol*. 2017;2:eam8929.

51. Scott NR, Swanson RV, Al-Hammadi N, et al. S100A8/A9 regulates CD11b expression and neutrophil recruitment during chronic tuberculosis. *J Clin Invest*. 2020;130:3098-3112.

52. Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome [published correction appears in *Lancet Respir Med*. 2020 Feb 25]. *Lancet Respir Med*. 2020;8:420-422.

53. Matute-Bello G, Downey G, Moore BB, et al. An official American Thoracic Society workshop report: features and measurements of experimental acute lung injury in animals. *Am J Respir Cell Mol Biol*. 2011;44:725-738.

54. Hong W, Yang J, Bi Z, et al. A mouse model for SARS-CoV-2-induced acute respiratory distress syndrome. *Signal Transduct Target Ther*. 2021;6:1.

55. Rezine F, Lesouhaitier M, Gregoire M, et al. SARS-CoV-2-induced ARDS associates with MDSC expansion, lymphocyte dysfunction, and arginine shortage. *J Clin Immunol*. 2021;41:515-525.

56. Rejige M, Dunet V, Letovance I, et al. Pulmonary lymphangitic carcinomatosis: diagnostic performance of high-resolution CT and $^{18}$F-FDG PET/CT in correlation with clinical pathologic outcome. *J Nucl Med*. 2020;61:26-32.

57. Yap GLR, Sachaphibulkij K, Foo SL, Cui J, Fairhurst AM, Lim LHK. Annexin-A1 promotes RIG-I-dependent signaling and apoptosis via regulation of the IRF3-IFNAR-STAT1-IFIT1 pathway in A549 lung epithelial cells. *Cell Death Dis*. 2020;11:463.

58. Besch R, Poeck H, Hohenauer T, et al. Proapoptotic signaling induced by RIG-I and MDA-5 results in type I interferon-independent apoptosis in human melanoma cells. *J Clin Invest*. 2009;119:2399-2411.

59. Vogl T, Ludwig S, Goebeler M, et al. MRp8 and MRp14 control microtubule reorganization during transendothelial migration of phagocytes. *Blood*. 2004;104:4260-4268.

60. Raquel MA, Aneriz N, Rouleau P, Tessier PA. Blockade of antimicrobial proteins S100A8 and S100A9 inhibits phagocyte migration to the alveoli in streptococcal pneumonia. *J Immunol*. 2008;180:3366-3374.

61. Kaplan RN, Rafii S, Lyden D. Preparing the “soil”: the premetastatic niche. *Cancer Res*. 2006;66(23):11089-11093.

62. Deguchi and Maru, unpublished results.

63. Pan S, Hu Y, Hu M, et al. S100A8 facilitates cholangiocarcinoma metastasis via upregulation of VEGF through TLR4/MyD-2 pathway activation [published correction appears in *Int J Oncol*. 2020 Apr;56(4):1046]. *Int J Oncol*. 2020;56(1):101-112.

64. Dexamethasone reduces death in hospitalised patients with severe respiratory complications of COVID-19. https://www.ox.ac.uk/news/2020-06-16-dexamethasone-reduces-death-hospitalised-patients-severe-respiratory-complications. Accessed May 21, 2021

65. RECOVERY Collaborative Group, Horby P, Lim WS, et al. Dexamethasone reduces death in hospitalised patients with COVID-19. *N Engl J Med*. 2021;384(8):693-704.

66. Dexamethasone in Hospitalized Patients with Covid-19. https://www.ox.ac.uk/news/2020-06-16-dexamethasone-reduces-death-hospitalised-patients-severe-respiratory-complications. Accessed May 21, 2021

67. Ruan SY, Lin HH, Huang CT, Kuo PH, Wu HD, Yu CJ. Exploring the heterogeneity of effects of corticosteroids on acute respiratory distress syndrome: a systematic review and meta-analysis. *Crit Care*. 2014;18:R63.

68. Japan's Eisai to launch clinical trial for coronavirus treatment. https://asia.nikkei.com/Spotlight/Coronavirus/Japan-s-Eisai-to-launch-clinical-trial-for-coronavirus-treatment. Accessed May 21, 2021

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