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Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Treating exuberant, non-resolving inflammation in the lung; Implications for acute respiratory distress syndrome and COVID-19

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ARTICLE INFO

Available online xxxxx

Keywords:

ARDS
COVID-19
Inflammation
p38 MAP kinase
SARS-CoV-2

ABSTRACT

While COVID-19, the disease driven by SARS-CoV-2 has ignited interest in the host immune response to this infection, it has also highlighted the lack of treatment options for the damaging inflammatory responses driven by pathogens that precipitate the acute respiratory distress syndrome (ARDS). With the global prevalence of SARS-CoV-2 and the likelihood of a second winter spike alongside seasonal flu, the need for effective and targeted anti-inflammatory agents is even more pressing. Here we discuss the aetiology of COVID-19 and the common signalling pathways driven by SARS-CoV-2, namely p38 MAP kinase. We highlight that p38 MAP kinase becomes elevated with increasing age, thereby driving many of the inflammatory pathways that precipitate death in old people with the added drawback of impairing vaccine efficacy in this susceptible age group. Finally, we review drugs available to inhibit p38 MAP kinase, their risks-versus-benefits as well as suggested dosing regimen to combat over-exuberant innate immune responses and potentially reverse vaccine inefficacy in older patients.

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1. Background

In the United Kingdom, between 10,000-25,000 people die/per year from seasonal flu infection. While the flu season spans about six months of the year, SARS-CoV-2 has resulted in 50,427 excess deaths in England,

contributing between 10.4% and 100% of excess mortality depending on place of death (Public Health England, PHE). At the time of writing, the worldwide death toll has surpassed a million and continues unabated. Certainly, vaccines and anti-viral agents combat flu and remdesivir is showing some efficacy against SARS-CoV-2 (Beigel et al., 2020).

Abbreviations: ARDS, Acute respiratory distress syndrome; Ang, Angiotensin; ACE, Angiotensin converting enzyme; COPD, Chronic obstructive pulmonary disorder; COVID-19, Coronavirus disease 2019; IFN, Interferon; IL, Interleukin; LPS, Lipopolysaccharide; MAP kinase, Mitogen-activated protein kinase; NETs, Neutrophil extracellular traps; PHE, Public Health England; SASP, Senescence-associated secretory phenotype; SARS-CoV, Severe acute respiratory syndrome coronavirus; TNF, Tumour necrosis factor.

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<https://doi.org/10.1016/j.pharmthera.2020.107745>

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Please cite this article as: D.W. Gilroy, R.P.H. De Maeyer, M. Tepper, et al., Treating exuberant, non-resolving inflammation in the lung; Implications for acute respiratory distr..., *Pharmacology & Therapeutics*, <https://doi.org/10.1016/j.pharmthera.2020.107745>

However, a significant clinical challenge arises from the exuberant and non-resolving inflammatory response triggered by these respiratory infections. This has become particularly apparent with SARS-CoV-2 and the unique pathologic sequelae that this infection triggers. In essence, despite the prevalence and pervasiveness of ARDS triggered by infectious stimuli, no therapeutic intervention exists to counterbalance the associated detrimental immune response. The United Kingdom NIHR-funded RECOVERY (Randomised Evaluation of COVid-19 thERapY) trial has found that dexamethasone reduced the risk of dying by one-third in ventilated patients and by 20% in patients receiving oxygen only (The RECOVERY Collaborative Group et al., 2020). There was no benefit among those who did not need respiratory intervention. With dexamethasone being a potent anti-inflammatory agent, these findings underline that the pathological sequelae triggered by SARS-CoV-2 infection are largely immune mediated. While the effects of dexamethasone on COVID-19 patients are heartening, there is scope to build upon this clinically meaningful success to improve outcomes for a broader infected population with urgent unmet clinical needs.

Arising from our growing understanding of the inflammatory response seen in COVID-19, we suggest that p38 MAP kinase is a tractable target for inhibiting the underlying pathology driven by SARS-CoV-2 and indeed other respiratory viral infections. By associating the pathogenesis of COVID-19 with elevated p38 MAP kinase activity we will draw strong similarities with immune senescence (Nikolich-Žugich, 2018). The term immune senescence refers to a state of immune dysfunction seen in older people. Two key features of immune senescence are the increased attraction of monocytes to inflamed tissues and their acquisition of a dysregulated pro-resolution phenotype as well as the senescence-associated secretory phenotype (SASP), a phenomenon where the immune system of older people generates more cytokines and clotting factors. We will argue that this may explain why older individuals with COVID-19 and other respiratory viral infections have significantly higher mortality rates than younger individuals. Finally, we will discuss the potential limitations of developing a vaccine to SARS-CoV-2 and flu, particularly in older people and suggest how a specific p38 MAP kinase inhibitor, at the point of vaccination, may enhance vaccine efficacy in COVID-19 infected patients.

2. The pathogenesis of COVID-19

In principle, there are several approaches we can use to protect ourselves from SARS-CoV-2. These include (1) develop a vaccine to re-programme our immune system against the virus, (2) develop an antiviral agent including antibodies/human convalescent serum and (3) deal with the deadly overexuberant immune response and pulmonary inflammation to the virus.

Currently there are numerous clinical trials ongoing to develop a treatment for patients infected with SARS-CoV-2. In no particular order, these include antiviral drugs, immune boosters as well as anti-inflammatory agents including anti-IL-1, anti-IL-6, mucolytics, anticoagulants, vasodilators, anti-angiogenesis as well as an antioxidant (α -lipoic acid), an antibiotic (azithromycin), and an anti-parasitic drug (Levamisole). As of April 2020, a novel therapeutic approach in the form of a Bruton Tyrosine Kinase inhibitor, acalabrutinib has been initiated to target the hyper-inflammatory response and resulting cytokine storm associated with COVID-19 infection in severely ill patients [NCT04346199] (Roschewski et al., 2020).

While the above largely target single pathways, from our perspective, the therapeutic ploy in preventing COVID-19 and flu-related deaths is to look at the pathobiology of the host response more holistically. So far, we have learned that a cytokine storm, including TNF, IL-6 and IL-2R α generation; clotting disorder/micro emboli; pronounced monocyte influx and oedema leading to failed inflammatory resolution are cardinal signs of severe COVID-19 pathogenesis (Giamarellos-Bourboulis et al., 2020; Meftahi, Jangravi, Sahraei, & Bahari, 2020; Moore & June, 2020).

Airway neutrophilia is increased during respiratory viral infections, and in combating the pathogenic insult, neutrophils generate neutrophil extracellular traps (NETs) that can precipitate NETopathic lung inflammation (Uddin, Watz, Malmgren, & Pedersen, 2019). Elevated NETs and serum levels of NET-associated products, including MPO-DNA complexes and citrullinated histone H3 in hospitalised patients with COVID-19 has been reported (Barnes et al., 2020; Zuo et al., 2020). Moreover, the extent of neutrophilia is a strong predictor of outcome in patients with COVID-19 (D. Wang et al., 2020; J. Wang et al., 2020). Thus, dysregulation of neutrophil effector functions provoked by SARS-CoV-2 infection provides an important contribution to the pathobiology of COVID-19.

In addition, it is clear that the older one is, the greater the likelihood that one will succumb to infection. In England, 90% of all COVID-19 related deaths occur in those over the age of 60 (36,961 out of 39,829 deaths as of July 7th, source: PHE). Indeed, the mortality rate in the over 60s is 16 times higher than in those under 60 (35% in ≥ 60 compared to 2% <60, source: PHE). Furthermore, a recent UK study on more than 17 million patients has identified in-hospital mortality from SARS-CoV-2 infections was strongly associated with increasing age (The OpenSAFELY Collaborative et al., 2020). Older patients were disproportionately affected by COVID-19 with the ≥ 80 years age group having major prognostic implications. In certain older patients, this may be attributable to an exaggerated immune response (cytokine storm) leading to the development of ARDS requiring assisted ventilation with subsequent multi-organ failure and ultimately death. It should be noted that pre-existing age-related co-morbidities such as hypertension, diabetes, obesity and cardiovascular disease play a role in the severity of COVID-19 in the elderly (Casucci, Acanfora, & Incalzi, 2020). As investigative immunopathologists, we suggest that one common molecular master switch that links each of these disease pathways to old people, is p38 mitogen-activated protein kinase (MAP) kinase. The p38 MAP kinases are highly conserved proline-directed protein kinases composed of α , β , γ and δ , isoforms which are encoded by distinct genes. The p38 α and p38 β isoforms are expressed ubiquitously and have been best studied to date (Cuenda & Rousseau, 2007).

3. p38 MAP kinase and immunosenescence

Immunosenescence refers to the age-acquired changes to the innate and adaptive immune system affecting our ability to efficiently respond to infections as well as develop long term immune memory, especially vaccinations (Nichol, Margolis, Wuorenma, & Sternberg, 1994; Pawelec & McElhane, 2020). Naïve B cell and T cell numbers, antigen presentation by dendritic cells and cellular proliferation are all negatively affected (Nikolich-Žugich, 2018). In addition to arrested growth and altered function, senescent cells, including not only haematopoietic but also epithelial and endothelial cells as well as adipose tissue show widespread changes in gene expression. These changes include the secretion of numerous proinflammatory cytokines, chemokines, growth factors, clotting factors and proteases, a feature termed the senescence-associated secretory phenotype (SASP) (Coppé, Desprez, Krtočila, & Campisi, 2010). The SASP has powerful endocrine activities, the evolutionary benefit of which has been suggested to prevent the development of cancer and to promote tissue repair or regeneration following injury. Importantly, p38 MAP kinase activity is both necessary and sufficient for development of SASP (Alimbetov et al., 2015; Freund, Patil, & Campisi, 2011). Finally, the SASP is mainly driven by the alpha isoform of p38 MAP kinase, as blocking this particular isoform resulted in SASP inhibition (Freund et al., 2011).

IFNs are not typically associated with the SASP and in fact are found to decline with age (Pillai et al., 2016; Prakash, Agrawal, Cao, Gupta, & Agrawal, 2012; Qian et al., 2011). Delayed type 1 IFN responses in murine SARS-CoV infection models exhibit an accumulation of inflammatory monocytes and macrophages in the lung, resulting in vascular leakage, increased lung inflammation and impaired virus-specific T

cell immunity. Depletion of these inflammatory myeloid cells protects mice from infection and prevents the lethal pneumonia that is otherwise observed (Channappanavar et al., 2016). Interestingly, decreased IFN γ expression in CD4⁺ T cells are associated with severe COVID-19 (Channappanavar et al., 2016). We do not know why p38 MAP kinase becomes elevated with age driving SASP or whether it directly or indirectly dampens IFNs.

At the downstream level, p38 MAP kinase activation is associated with induction of transcription factors, such as c-Fos which coordinate the induction of pro-inflammatory mediator expression (Frödin & Gammeltoft, 1999). The disease relevance of this signalling pathway in regulating immune responses in the context of COVID-19 disease was reported in a recent study. The blood expression profile of c-Fos was markedly upregulated in established COVID-19 infected patients but found to be downregulated in individuals undergoing disease recovery (L. Huang et al., 2020). These findings suggest that regulation of the p38 MAP kinase pathway could serve as an auto-regulatory mechanism, therefore limiting ensuing cytokine storm and thereby prompting resolution in COVID-19 infected patients and a return to homeostasis. It is unclear to what extent this p38 MAP kinase regulation of immune responses in COVID-19 patients is influenced by age.

Consequently, we hypothesise that the underlying pathologies driven by SARS-CoV-2 and that manifest more profoundly in older (>60 years) COVID-19 patients are a consequence of p38 MAP kinase overexpression or enzymatic activity. For instance, p38 MAP kinase inhibitors dampen the synthesis of pro-inflammatory cytokines and would therefore block the lethal COVID-19-induced cytokine storm (Panigrahy et al., 2020). p38 MAP kinase inhibitors can also block the degree of sputum neutrophilia and levels of neutrophil-promoting factors in the airways, such as TNF- α that is important in regulating persistent neutrophilic lung inflammation (Patel et al., 2018). p38 MAP kinase inhibitors improve vasodilation/vascular dysfunction, prevent clotting, and inhibiting viral replication (Banerjee, Narayanan, Mizutani, & Makino, 2002; Cheriyan et al., 2011; Klemm et al., 2017; Koch et al., 2012; Sakurai et al., 2004; P. Shi et al., 2017). Indeed, multiple viruses including SARS-CoV and influenza activate p38 MAP kinase as part of their life cycle suggesting that targeting p38 MAP kinase will not only influence the host response but might in fact block viral action itself (Börgeling et al., 2014; Kopecky-Bromberg, Martinez-Sobrido, & Palese, 2005). In addition, from our recent work we found that the immune system of old people takes longer to resolve compared to younger counterparts (De Maeyer et al., 2020). We reported that older people have high levels of monocyte p38 MAP kinase phosphorylation, recruit more monocytes to sites of inflammation with these cells losing their ability to efferocytose immune debris, a key determinant of inflammatory resolution. As a result of dysfunctional efferocytosis, inflammatory monocytes do not acquire a resolution phenotype, but are more M1-like thereby generating Th-1 like cytokines (De Maeyer et al., 2020).

SARS-CoV-2 infects lung cells by interacting with angiotensin (Ang) converting enzyme (ACE)2 essentially downregulating this marker in lung epithelium (Glowacka et al., 2009; Kuba et al., 2005; Zhang, Penninger, Li, Zhong, & Slutsky, 2020). Work has shown that ACE2 and its enzymatic products Ang(1-7) protect the lung from injury and subsequent inflammation, whereas increased ACE1 can skew AngII signaling towards adverse effects and excessive inflammatory damage (Meng et al., 2014; Sriram & Insel, 2020; L. Wang, Wang, Yang, Guo, & Sun, 2015). Bleomycin-induced lung damage models in rats have shown this is in part due to blockade of MAP kinases by ACE2 activity (Meng et al., 2014). Conversely, AngII can downregulate ACE2 via activation of p38 MAP kinase resulting in ACE1/ACE2 imbalance (Koka et al., 2008), whereas p38 MAP kinase blockade using SB203580 results in overexpression of ACE2 in lung fibroblasts *in vitro* (Meng et al., 2014). It is possible, therefore, that targeting p38 MAP kinase could help to restore ACE1/ACE2 balance in the lung, counteracting fibrosis and inflammation, and ultimately improving outcome.

Another mechanism of interest is the upregulation of pyroptosis, a process of inflammatory cells death associated with old age, which has been shown to occur in cultured macrophages challenged with SARS-CoV proteins (Chen, Moriyama, Chang, & Ichinohe, 2019; C.-S. Shi, Nabar, Huang, & Kehrl, 2019). This occurred via activation of the inflammasome, a process that has been shown to be amenable to p38 MAP kinase regulation in murine models of acute lung injury (D. Li, Ren, Jiang, & Zhu, 2018). It is becoming apparent that blood lactate dehydrogenase levels, a key pyroptosis-related protein early in COVID-19 disease correlates with severe disease progression (Y. Huang et al., 2020; Wynants et al., 2020; Xiang et al., 2020; Zhao et al., 2020). Interestingly, we found in experimental skin blister that LDH is increased in those of our older volunteers in a manner that was reversed following p38 MAP kinase inhibition (De Maeyer et al., 2020).

Taken together, with earlier reports demonstrating that p38 MAP kinase is activated during lung aging with a corresponding increase in pro-inflammatory cytokines (Z. Li et al., 2011; Ren et al., 2014), we argue that p38 MAP kinase is a master regulator of many of the negative hallmarks of COVID-19. Indeed, infection of Vero E6 cells by SARS-CoV induced phosphorylation of p38 MAP kinase as well as its downstream targets including MAPKAPK-2, HSP-27, CREB, and eIF4E; events that were reversed using the p38 MAP kinase inhibitor, SB203580 (Mizutani, Fukushi, Saijo, Kurane, & Morikawa, 2004). More recently, phospho-proteomic analysis revealed that p38 MAP kinase is activated in both Vero E6 and ACE2-A549 human lung carcinoma cells infected with SARS-CoV-2 resulting in cytokine production and cell cycle arrest. p38 inhibition, using SB203580, decreased both viral replication and inflammatory cytokine production in a dose-dependent manner (Bouhaddou et al., 2020). The effects of p38 MAP kinase inhibition on cell cycle arrest was not evaluated, but a link between the two exists and could play a role in SARS-CoV-2 infection (Bouhaddou et al., 2020; Lee et al., 2002; Yee et al., 2004).

Consequently, its inhibition will have more profound effects on the disease severity and duration than targeting single pathways. More importantly, as p38 MAP kinase becomes elevated with age and as the pathogenesis of COVID-19 reflects an overactive p38 MAP kinase, we suggest that acute inhibition of p38 MAP kinase would be more effective in old people, the high-risk cohort that are most likely to die if infected with SARS-CoV-2.

4. Will a vaccine benefit the most vulnerable – old people?

There is the expectation that a vaccine against SARS-CoV-2 will be developed. The problem is that vaccines lose their efficacy in older people. For instance, the flu vaccine is 80% effective in 25-50 years old but is only 40% effective in people over 75 (Goodwin, Viboud, & Simonsen, 2006; Nichol et al., 1994; Pawelec & McElhaney, 2020); the very patient group that is predominantly dying in response to SARS-CoV-2 infections. The therapeutic challenge, therefore, is to boost vaccine efficacy in older patients. It has been postulated by our group that too much, or inappropriate inflammation, generated by the SASP, may impair the development of optimal host immunity as we age. Indeed, p38 MAP kinase inhibition was shown to restore antigen-specific, T cell mediated immunity in older subjects *in vivo* (Vukmanovic-Stejic et al., 2017). Therefore, short-term inhibition of p38 MAP kinase will enhance rather than inhibit immunity *in vivo*. In essence, we have always contended that inflammation arising from senescent cell and monocyte interactions is associated with immune decline during ageing and can be reversed. Importantly, this reversal can be achieved by concomitant administration of a p38 MAP kinase inhibitor alongside vaccines, which will arguably result in greater efficacy in old people.

5. Side effects of p38 MAP kinase inhibition

In addition to immune responses, there is experimental evidence across several organ systems suggesting that p38 MAP kinase also

mediates developmental, differentiation and proliferation processes (Martínez-Limón, Joaquin, Caballero, Posas, & Nadal, 2020). Therefore, as a consequence of the wide-ranging regulatory role of p38 MAP kinase in diverse cellular processes, the possibility of adverse events resulting from undesired pharmacological activity is a concern for the p38 MAP kinase inhibitor drug class. However, p38 MAP kinase inhibitors have been administered to patients with rheumatoid arthritis, Crohn's disease and chronic obstructive pulmonary disorder (COPD) for as long as 12 weeks (Fisk et al., 2018; Genovese et al., 2011; Schreiber et al., 2006). Whilst treatment-emergent adverse events have been reported that include dizziness and skin rash, these drugs are generally well tolerated.

Certainly, clinical development of orally active p38 MAP kinase inhibitors have flagged safety signals, probably due to "off target" effects on other enzymatic pathways resulting in dose-limiting toxicity (Dominguez, Powers, & Tamayo, 2005; Fisk, Gajendragadkar, Mäki-Petäjä, Wilkinson, & Cheriyan, 2014; Genovese et al., 2011). Avoidance of off-target effects have been the primary safety concerns, hence spurring the development of inhalable p38 MAP kinase inhibitors to minimise side effects by reducing systemic exposure. In a recent phase I study, inhaled administration of the specific inhibitor of p38 α MAP kinase (AZD7624) reduced the raised neutrophil numbers in induced sputum in response to LPS challenge in normal volunteers. The incidence of adverse events reported was similar across both treatment groups, and the most commonly reported adverse event was upper respiratory tract infection (Patel et al., 2018; Pehrson et al., 2018).

In the phase II stage of the trial, inhaled AZD7624 failed to show any clinical benefit in patients with COPD. Although AZD7624 was well tolerated, the reported incidence for the majority of adverse events was higher for participants in the active treatment group compared with placebo (Patel et al., 2018). The most commonly reported adverse events in this trial was indeed COPD, followed by dyspnoea and cough. There was no evidence of AZD7624-related hepatotoxicity or rash, unlike with orally active p38 MAP kinase inhibitors (Dominguez et al., 2005; Fisk et al., 2014; Genovese et al., 2011).

More specific p38 MAP kinase inhibitors were well-tolerated in a studies of COPD patients displaying an adequate safety profile but resulting in increased drug-related adverse events compared to placebo (Pascoe et al., 2017; Watz, Barnacle, Hartley, & Chan, 2014). In a phase II trial of the oral p38 α MAP kinase inhibitor, PH-797804 given to patients with COPD was shown to demonstrate improvements in lung function parameters and dyspnoea relative to placebo.

However, rash was the most frequently reported treatment-related adverse event in that study (MacNee, Allan, Jones, Salvo, & Tan, 2013). A larger clinical trial of another oral p38 α inhibitor, losmapimod (GW856553), failed to improve any clinical outcomes of exercise tolerance or lung function in patients with COPD. Although losmapimod was well tolerated and showed no evidence of hepatotoxicity, the most common treatment-emergent adverse event observed was dose-related dermatological reactions (pruritus) (Watz et al., 2014). Indeed, in another study systemic dilmapimod was well tolerated in severely injured subjects at risk of ARDS. At the dose levels tested in this study, there was no evidence of hepatotoxicity in trauma patients (Christie et al., 2015); this is an important finding given that development of other orally active p38 MAP kinase inhibitors has been discontinued because they have caused an increase in liver transaminases.

6. Treatment strategy of COVID-19 with p38 MAP kinase inhibitors

The recent success of the RECOVERY trial highlights the tractability of targeting overexuberant immune responses in critically ill COVID-19 patients. However, such a blunt approach might work in some but not others and the multifactorial nature of this infectious disease needs to be accounted for. Recently, it has been suggested that we should aim to achieve the optimal "Goldilocks level of immune activity" in patients, where hyperimmune responses are inhibited, but not to the point that viral spread becomes an issue. Indeed, given how p38 MAP kinase is involved in regulating these processes, we believe that p38 MAP kinase blockade might be able to effectively target the damaging virally driven inflammation observed. The advantage of p38 MAP kinase is that in addition to oral inhibitors that have been developed over the years, there are also inhaled p38 MAP kinase inhibitors for local delivery into the lung (Pehrson et al., 2018). Given that the respiratory mucosa is one of the main portals of entry for SARS-CoV-2 and consequently, the pathology of COVID-19 initiates in the lung, then local delivery represents a logical and potentially very efficacious approach. Indeed, this could be accompanied with administration of an oral or parenteral p38 MAP kinase inhibitor to counter-regulate any cytokine storm spill over, thereby tackling the disease both locally and systemically. Examining the temporal profile of pathological events driven by SARS-CoV-2 infection, it is clear that most phases of the disease will benefit from acute p38 MAP kinase inhibition (Fig. 1). Therefore, administering a p38 MAP kinase inhibitor, especially in combination with an anti-viral

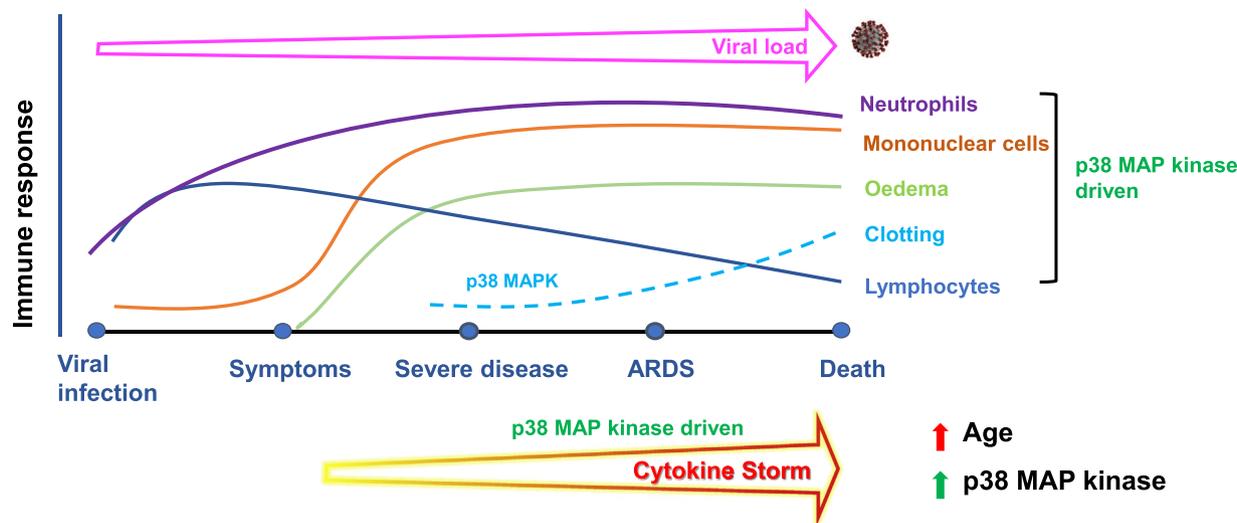


Fig. 1. Time course of immune activation following SARS-CoV-2 viral infection in the context of p38 MAP kinase activation. In essence, many of the pathological events underpinning mortality following SARS-CoV-2 infection including neutrophilic and monocytic infiltrates, cytokine storm, oedema formation and clotting as well as risk factors are driven by elevated p38 MAP kinase activity.

agent at onset of symptoms (usually with seven days of infection) will immediately tackle viral load. If symptoms are ignored by the infected individual or rapidly progress as in old people or people with multimorbidities, then the ensuing cytokine storm, and viral load, will be dampened by p38 MAP kinase inhibition. Indeed, coagulation and pulmonary oedema will also be inhibited by continued p38 MAP kinase inhibition. Thus, the relatively well-defined disease trajectory has suggested that most phases of COVID-19 will benefit from the acute inhibition of p38 MAP kinase; however, the sooner it is administered, the better.

7. Conclusion

We propose the acute inhibition of p38 α MAP kinase for adjunct treatment of COVID-19 as many of the pathogenic processes that have been thus far associated with death, including cytokine storm, clotting, oedema and pulmonary inflammation are driven by this signalling pathway. Of benefit, is the combination of a local as well as systemic p38 α MAP kinase inhibitor delivery that over the course of an acute treatment regimen (days rather than weeks) would be expected to carry a low/no burden of side effects. If, however, inhibiting p38 MAP kinase compromised host defence during infection-driven acute respiratory distress syndromes, such prior knowledge, in light of an effective treatment, at least pre-warns of appropriate prophylactic antibiotic/antifungal treatment.

Declaration of Competing Interest

DWG and MT have equity ownership in Senex Therapeutics Inc. MU is an employee of AstraZeneca and holds share in the company. The other authors declare no competing interests.

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