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Perspective

COVID-19 lockdown: de-risking exit by protecting the lung with leukaemia inhibitory factor (LIF)

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ABSTRACT

There are two key needs in COVID-19 management: (i) to reduce SARS-CoV-2 viral infection rate; and (ii) to reduce death rate of those infected - the subject of this commentary. The current WHO estimated global mortality rate is 3.4% (March 2020) and the global death toll has now past 200,000 (April 2020). Without therapy the COVID-19 pandemic is escalating exponentially: from the first reported death in Wuhan China 10th January 2020, it took 91 days for the global death toll to pass 100,000 - then a further 16 days to reach 200,000. A vaccination program will take 1–2 years to roll out, once safety and efficacy is proven. Anti-virals are being sought mainly amongst repurposed drug candidates but also with combinatorial screening of libraries, for example to block virus binding angiotensin converting enzyme 2 (ACE2) - ACE2 providing the receptor on cells that allows viral entry. Cell-based approaches include stem cells and exosomes but these will never meet scale of need whilst also carrying risk of viral transmission if contaminated. Countries have introduced Population control with social distancing and lockdown to isolate individuals: this has reduced infectivity rate - "R" - where R denotes the average number of people an infected person will spread the illness to. But, after lockdown, the virus remains: the probability of R increasing again is high.
The new danger is exit from lockdown. Here, leukaemia inhibitory factor (LIF) represents an untapped resource to boost the lung's own resistance to developing COVID-19 - reducing risk of severe disease as nations cautiously leave lockdown to return to normality.

1. Introduction

As the COVID-19 pandemic unfolds, analysis of fatalities reveals a profound age-related risk across the whole spectrum of COVID-19 disease. [1] The exceptionally low (0.2%) risk of death for young people increases ten-fold by the age of 60. So, what is protecting the young who are infected with the SARS-CoV-2? Is there a specific factor? Or is it simply a feature of youth and lack of co-morbidities, such as diabetes and high blood pressure?

The COVID-19 pandemic illustrates just how vulnerable humans are to a virulent viral infection when it is in the lung. That we have survived at all might be thanks to a single biological growth factor - called LIF. LIF is a stem cell growth factor that remains active throughout life, but slowly diminishes with age. In the lungs, LIF has evolved to control inflammation and at the same time repair co-lateral damage to tiny blood capillaries and the alveoli - delicate air sacs where oxygen exchange occurs.

Huang et al. [2] then Zhou et al. [3] were the first to report on COVID-19 mortality rate. From Wuhan, China, their studies presented the epidemiological, clinical, laboratory, and radiological characteristics, together with treatment and clinical outcomes of these patients. They found a high trajectory of disease progression, with only 3.5 days between hospital admission and need for intensive care - a dramatic alert to the world on how quickly the patient with severe COVID-19 might succumb (Fig. 1).

1.1. LIF and the Lung

The requirement for LIF to protect the lung during infection - first reported in 1994 [4] and later confirmed and expanded [5–7] is of profound importance. The model below is the author's own, aiming to explain in the context of COVID-19 how the lung engages with LIF to protect lung function after viral entry into the lung's alveoli, as depicted in Fig. 2.

- The pulmonary alveolar epithelium is a single-cell-thick layer, one side covered by surfactant and exposed to the air within the lung, and the other with an extracellular matrix surrounded by capillaries where gaseous exchange happens.
- Alveolar macrophages are resident within the alveolus.
- Alveolar Type I epithelial cells (0.2 μ thick) - cover more than 95% of the alveolar surface: these cells are vulnerable to dying during inflammation but are unable to self-renew.
- Alveolar Type II epithelial cells (0.6 μ thick) - are numerically dominant and secrete surfactant at the blood-air barrier to retain patency of the alveolar structure to air.

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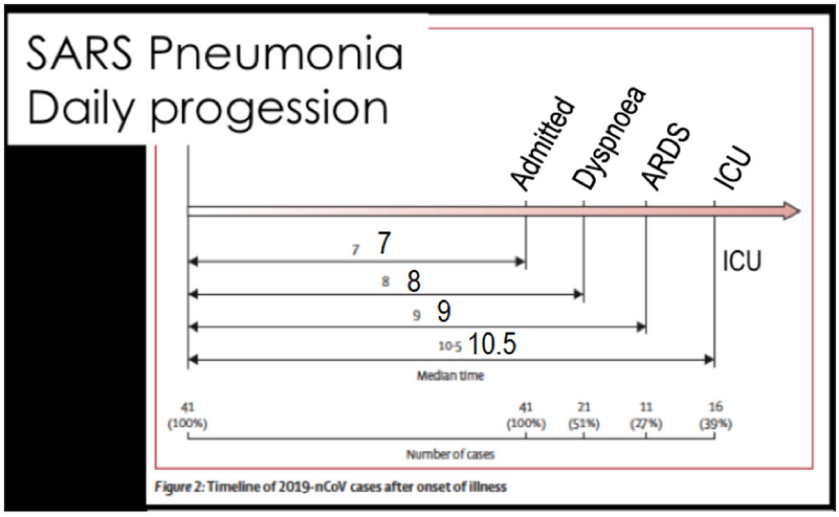

Fig. 1. Wuhan China report on SARS Pneumonia Daily Progression: adapted from Huang et al. Lancet 24th January 2020. Axis indicates number of patients followed with percentage of these progressing to increasingly severe disease, against the timeline from infection.

- Stem Cell Niche: the Type II cells also represent a stem cell niche capable of cell division and differentiation into either Type I or Type II cells - so regenerating the delicate alveolar structure. These Type II cells are the source of LIF - not only reflecting the "stemness" property of Type II cells, but also providing rapid release of tissue-protective LIF in response to infection.
- During Pneumonic Infection, alveolar macrophages are triggered to release inflammatory cytokines: analysis of LIF in response to infection revealed that all inflammatory cytokines induce LIF expression - with the one exception - that being IL-6. IL-6 directly suppresses LIF [9,10] explaining the potency of IL-6 in driving cytokine storm once IL-6 becomes dominant. Note also that IL-6 up-regulates vascular angiotensin type 1 receptor with propagation of oxidative stress and vascular endothelial dysfunction [11].
- Angiotensin-converting enzyme 2 (ACE-2) - the receptor for SARS-CoV-2 [12,13] - is highly expressed on Type II epithelial cells. Here the model predicts that - following infection - cytokines released from the alveolar macrophages trigger Type II cells to produce LIF.
- Myeloid-epithelial cross-talk was first demonstrated by Quinton's laboratory [8]. The discrete cell lineages coordinate synthesis of tissue-protective LIF during pneumonia: maximal expression of pulmonary epithelial-derived LIF (Type II cells) was dependent on myeloid cells (alveolar macrophages) - revealing a co-operative axis essential to resist severe disease and ARDS.
- Compounding properties of LIF operate - (i) reparative and regenerative, acting on the stem cell niches of the Type II alveolar epithelial cells to maintain alveolar physiology and structure for gaseous exchange. (ii) Control against excessive inflammatory cascade and cytokine storm, where LIF is anti-inflammatory and directly opposes IL-6-driven immunity. (iii) Also LIF is required to protect vascular integrity from inflammation-induced redox damage that may trigger vascular leak and eventual predisposition to sepsis. Overall, LIF's hyper-acute protective responses become progressively reinforced by homeostatic recovery from the primary inflammation triggered by virulent SARS-CoV-2 infection: without LIF, or with insufficient LIF, progression to inflammatory COVID-19 occurs.

1.2. Why does LIF decrease with Age? Can LIF-activity be rejuvenated?

A LIF/IL-6 axis exists [9,10] - permissive for vital self-tolerant functions to coexist with vital aggressive immunity against pathogens. Mechanistically, the LIF/IL-6 axis is a simple binary switch first discovered in T lymphocytes operated by LIF (quiescence - Treg) versus IL-6 (inflammation - TH17). Crucially, LIF is able to directly oppose IL-6 activity - controlling against cytokine storm. But over time, background levels of circulating IL-6 gradually increase, tipping the balance between LIF and IL-6 towards inflammation, with reciprocal loss of LIF since IL-6 directly inhibits transcription of the LIF-receptor - in effect making cells blind to LIF [13]. This age-related shift is sometimes termed "inflammaging". Notably both IL-6 and age are linked to COVID-19 mortality [2,3]: here increasing IL-6, and decreasing LIF, explains why COVID-19 is so dangerous for the elderly.

Targeting IL-6: For severe-critical COVID-19 clinical approaches to reduce mortality include to target the IL-6 pathway - for example using the humanized monoclonal antibody Tocilizumab to bind and block the IL-6 receptor. Although with reported efficacy in rescuing ARDS patients [14], removal of IL-6 signaling will not recover the compounding values of LIF.

Prevention is better than cure - boosting LIF. The next question is - can LIF per se be used to treat COVID-19? Evidence suggests, yes. Recombinant LIF protein is potently protective against pneumonia in animal models where the LIF prevents fluid accumulation and respiratory distress that leads to death in pneumonia. Moreover, removal of LIF by treating with anti-LIF results in acute alveolar vascular leak as illustrated in the top right image of Fig. 2. The requirement for LIF to prevent ARDS in humans is evidenced by the rare genetic disease - Stuve-Wiedemann Syndrome [15] - with infant death linked to ARDS occurring around 3 months of age: here a mutation in the gene encoding gp190 - the LIF-specific receptor - prevents normal LIF signalling.

Learning from the host teaches that therapeutic LIF will tap into natural regulatory pathways to protect upstream against pathogenesis of COVID-19 progression, directly in contrast to removal of IL-6 as a downstream mediator of consequential pathophysiology. LIF actively up-regulates its own expression in an autocrine manner, inducing both LIF and gp190 - the LIF-specific receptor subunit. Thus therapeutic LIF can reset endogenous control of the LIF/IL-6 axis back to homeostasis, correcting the inflammaging shift. Moreover, by protecting the vascular endothelium, LIF not only protects against a chronic legacy of disease in the lung, but also reduces chance of other long-term chronic conditions developing in other organs post COVID-19 recovery.

1.3. A global perspective on urgency to exploit LIF to protect the inflamed lung

Synthetic LIF is a game changer - as the fight against COVID-19 gains ground and policy makers consider immunization strategies and anti-viral

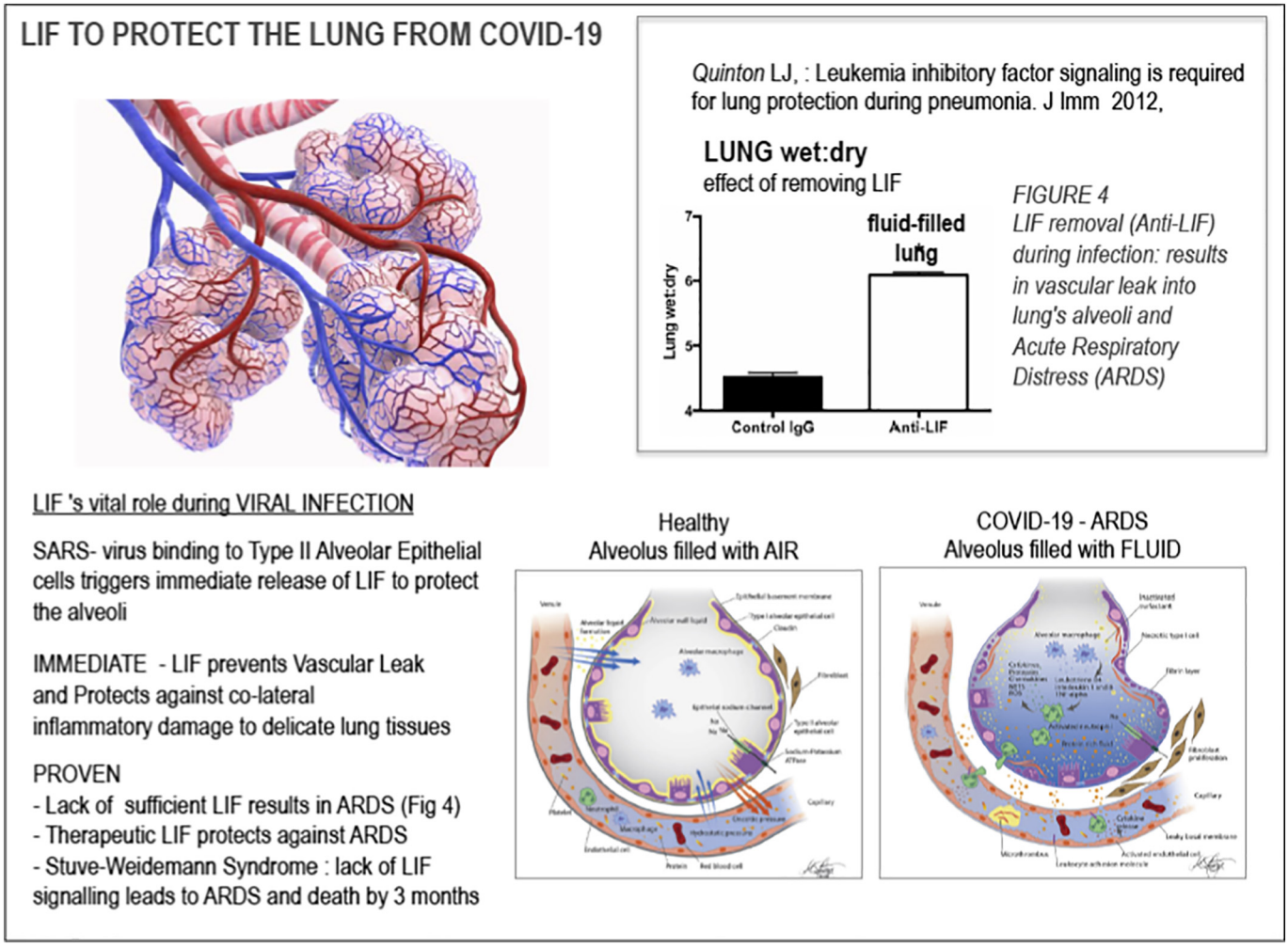

Fig. 2. *Top left:* Artist's impression of three clusters of alveoli of the lung (Google Images). *Bottom centre* (adapted from Reference [16]): Schematic of a healthy alveolus. The alveolar epithelium and capillary endothelium are intact. The characteristics of the pulmonary circulation and intact epithelial endothelial barrier allow for formation of the alveolar wall liquid (AWL) while maintaining the air-filled, fluid-free, status of the alveoli. The AWL facilitates gas exchange and is a medium for dispersal of surfactant and alveolar macrophages, which is essential for maintaining alveolar stability and host defenses. The intact sodium-dependent vectorial transport across type II alveolar epithelial cells regulates the removal of excess alveolar fluid. *Bottom right* (adapted from Reference [16]): Schematic of pathophysiology in ARDS. There is a loss of epithelial and endothelial barrier integrity and loss of function leading to increased permeability pulmonary edema. Solutes and large molecules such as albumin enter the alveolar space. In the presence of proinflammatory mediators and activated endothelium, leukocytes traffic into the pulmonary interstitium and alveoli. There is activation of coagulation and deposition of fibrin in capillaries and alveoli with increased concentrations of fibrinogen and fibrin degradation products in the edema fluid. Surfactant depletion and degradation result in large increases in surface tension and loss of alveolar shape and integrity. Recovery is preceded by fibroblast proliferation. NETs, neutrophil extracellular traps; ROS, reactive oxygen species. *Top right:* Effect of removing endogenous LIF in a mouse model of pulmonary pneumonia (adapted from Reference [6]).

agents - the option of also boosting natural resistance against COVID-19 symptoms by treating with LIF could shift the dynamics of lockdown and exit strategies. The whole global economy will benefit - speeding up recovery and shifting current predictions of time to regain normality.

Moreover, the protective value of LIF is universal. As new SARS viruses emerge, immediate deployment of LIF will reduce impact and buy time for viral-specific immunization programs to be developed: the availability of LIF will - globally - protect infrastructure and the working economy. We know LIF works. It has evolved over millions of years to do the job of protecting breathing.

LIF meets the urgent healthcare need to reduce disease severity and, thus reduce pressure on the global healthcare systems. In practice, LIF can be delivered to the patient for example using a nebuliser - similar to an asthma “puffer”, but loaded with LIF for supply directly into the airways and lung tissues.

Alternatively, the LIF can be given intravenously where it will reach the lung within seconds, passing through the inflamed capillaries to quench the inflammation. Evidence from patients treated with stem cells - an alternative source of LIF - shows a rapid, critical shift wherein the inflammatory cells of the inflamed lung move out and back into the blood and start secreting anti-inflammatory growth factors, including natural LIF. Thus, therapy with LIF will reset the patient's own ability to produce LIF and recover lung function.

Clearly, in contrast to stem cells, vials of therapeutic LIF - with no limit on scale of manufacture and global distribution - provides a rapidly available treatment resource for COVID-19 pandemics - including in the third world that lacks advanced healthcare infrastructures. The route of delivery is simple - inhaled or intra-venous. LIF is also compatible with other treatments, such as ventilator-assisted breathing. At all levels, LIF provides a high value, low cost solution by naturally increasing resistance to severe COVID-19 disease.

Conflict of Interest

The authors declare no conflict of interest.

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