COVID-19: Lung-Centric Immunothrombosis

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The respiratory tract is the major site of infection by SARS-CoV-2, the virus causing COVID-19. The pulmonary infection can lead to acute respiratory distress syndrome (ARDS) and ultimately, death. An excessive innate immune response plays a major role in the development of ARDS in COVID-19 patients. In this scenario, activation of lung epithelia and resident macrophages by the virus results in local cytokine production and recruitment of neutrophils. Activated neutrophils extrude a web of DNA-based cytoplasmic material containing antimicrobials referred to as neutrophil extracellular traps (NETs). While NETs are a defensive strategy against invading microbes, they can also serve as a nidus for accumulation of activated platelets and coagulation factors, forming thrombi. This immunothrombosis can result in occlusion of blood vessels leading to ischemic damage. Herein we address evidence in favor of a lung-centric immunothrombosis and suggest a lung-centric therapeutic approach to the ARDS of COVID-19.

Keywords: acute respiratory distress syndrome (ARDS), coronavirus, COVID-19, cytokine storm, NET, SARS-CoV-2

INTRODUCTION

SARS-CoV-2, the coronavirus responsible for COVID-19, initially infects the nasal, bronchial and alveolar epithelial cells (Hoffmann et al., 2020; Milewska et al., 2020; Zhu et al., 2020). Resident immune cells such as macrophages and dendritic cells are also infected, albeit to a lesser extent (Carsana et al., 2020; Schaefer et al., 2020; Yang et al., 2020). The SARS-CoV-2 pulmonary tropism is manifested in the clinical features of COVID-19 (e.g., cough, dyspnea). The clinical course of SARS-CoV-2 infection is subdivided into three outcomes. Most patients will be asymptomatic or display minor respiratory symptoms and recover without hospitalization. Roughly 10-20% of affected individuals will advance to pneumonia/hypoxia and require hospitalization, but will eventually recover (Chowdhury et al., 2021; Morris et al., 2021). Only a small fraction (5 - 10%) of COVID-19 patients will progress to acute respiratory distress syndrome (ARDS) and require aggressive treatment in intensive care units (e.g., mechanical ventilation) (Chowdhury et al., 2021; Morris et al., 2021). Some of these patients will eventually succumb to the disease with the major cause of death being respiratory failure (Ackermann et al., 2020; Phua et al., 2020; Tay et al., 2020). Autopsy findings indicate that the lungs bear the greatest pathologic burden, characterized by diffuse alveolar damage, inflammatory cell infiltrates and thrombosis (Ackermann et al., 2020; Lax et al., 2020; Liu et al., 2020; Deshmukh et al., 2021). Of note, organs remote from the initial site of infection such as
the heart and kidneys are not spared. Histopathologic features in autopsy specimens of these remote organs include the presence of microvascular thrombi adjacent to regions of necrosis (Ackermann et al., 2020; Rapkiewicz et al., 2020; Tang et al., 2020). Thus, in severe COVID-19 there is evidence indicative of an hypercoagulative state and multiorgan dysfunction.

A dysregulated immune response to SARS-CoV-2 infection is believed to play a major role in the pathogenesis of COVID-19. Specifically, there is an impaired antiviral response in conjunction with an excessive inflammatory response (Blanco-Melo et al., 2020; Hadjadj et al., 2020). Lymphopenia is common and may be coupled to markers of T cell exhaustion (Blanco-Melo et al., 2020; Hadjadj et al., 2020). Lymphopenia in the circulation and decreased numbers in lymphoid tissues (Diao et al., 2020; Giamarellos-Bourboulis et al., 2020; Lee et al., 2020; Liu et al., 2020; Lucas et al., 2020; Zhao et al., 2020; Zheng et al., 2020; Ronit et al., 2021). An inadequate lymphocyte-mediated antiviral response would protract the viral infection and thereby exacerbate the inflammatory response (Lee et al., 2020; Lucas et al., 2020; Manjili et al., 2020; Ronit et al., 2021).

**LUNG-CENTRIC INFLAMMATION**

Analyses of bronchoalveolar lavage fluid (BALF) of COVID-19 patients indicate a pro-inflammatory environment within the lungs (De Biasi et al., 2020; Liao et al., 2020; Pandolfi et al., 2020; Wang et al., 2020a; Ronit et al., 2021). Their BALF is enriched in pro-inflammatory chemokines and cytokines as well as activated macrophages and neutrophils. Chemokines (e.g., IL-8) dominate the BALF profile in moderate cases of COVID-19, whereas cytokines (e.g., TNF-α, IL-6) are prevalent in more severe cases (Liao et al., 2020).

A major cytokine involved in initiation and progression of the inflammatory response is IL-1β, the activation of which is dependent on the NLRP3 inflammasome (Kelley et al., 2019; Swanson et al., 2019; Zhao and Zhao, 2020). In brief, detection of viral material (e.g., PAMPs) by alveolar macrophages activates the NFκB transcription pathway, resulting in the generation of nascent pro-IL-1β as well as components of the NLRP3 inflammasome (Figure 1). Subsequently, the inflammasome is assembled and serves as a platform for caspase-mediated cleavage of pro-IL-1β to the mature form. IL-1β lacks a signal sequence and is retained in the cytoplasm until an exit portal is created in the plasma membrane. To this end, gaseinms, also activated by the caspases, enter the plasma membrane and oligomerize to form pores (Broz et al., 2020). IL-1β and other pro-inflammatory material (e.g., DAMPs) exit via these gaseinm pores. Collectively, the released mediators amplify the local inflammatory response via feed-forward mechanisms, including cytokine-induced cytokine release and recruitment of additional innate immune cells (e.g., neutrophils).

The NFκB pathway and the NLRP3 inflammasome appear to be operative in COVID-19 patients (Lee et al., 2020; Hariharan et al., 2021) and may contribute to lethality (Lara et al., 2020). SARS-CoV-2 infects human monocytes and activates the NLRP3 inflammasome, resulting in gaseinm-mediated pyroptosis

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**FIGURE 1** | Schematic of lung-centric inflammation and immunothrombosis in response to SARS-CoV-2 infection. Resident alveolar macrophages mount an inflammatory response to infection of the lungs by SARS-CoV-2. Macrophages detect viral material e.g., pathogen-associated molecular patterns (PAMPs), PAMPs activate the NFκB pathway which generates pro-IL-1β and components of the inflammasome. Assembly and functional activation of the inflammasome results in caspase-mediated cleavage of pro-IL-1β to the mature form. IL-1β as well as other inflammatory mediators, such as damage-associated molecular patterns (DAMPs) exit the macrophage through the GSDMD pores. The inflammatory response is amplified by feed-forward mechanisms and recruitment of additional leukocytes, e.g., neutrophils. Activated neutrophils can extrude neutrophil extracellular traps (NETs), a meshwork of decondensed DNA decorated with granule-derived proteases and antimicrobials. Inflammammasome-derived caspase as well as granule-derived elastase activate GSDMD to form pores for NET release. NETs formed in the alveolar space can induce lung injury while NETs generated within blood vessels sequester platelets and coagulation factors to promote thrombogenesis. Modified from (Tall and Westerterp, 2019).
(Ferreira et al., 2021). Active caspases and cytokines are present in the circulation of these patients, with higher levels noted in severe cases (Rodrigues et al., 2021). Further, inflammasome components are detected in autopsy specimens of lung tissue, primarily in macrophages and to a lesser extent in alveolar epithelia (Rodrigues et al., 2021; Toldo et al., 2021).

**LUNG-CENTRIC “CYTOKINE STORM”**

The cytokines and chemokines generated within the lungs in response to infection can spill into the general circulation, a convenient sampling reservoir for both clinical and experimental purposes. Based on elevated circulating levels of cytokines, the term “cytokine storm” has been used as a descriptor of the hyperinflammatory response involved in the pathogenesis of COVID-19 (Barnes et al., 2020; Castelli et al., 2020; Faigenbaum and June, 2020; Mehta et al., 2020; Merad and Martin, 2020; Tay et al., 2020). This descriptor has been challenged on the grounds that the measured blood levels of inflammatory cytokines in COVID-19 patients are orders of magnitude less than levels reported in other cases of ARDS such as sepsis or influenza (Kox et al., 2020; Mudd et al., 2020; Sinha et al., 2020). The issue is further confounded by a lack of a precise definition for the term “cytokine storm” (Faigenbaum and June, 2020). A reasonable approach to avoid this rather semantic issue is to consider the local pulmonary storm as being more severe than the systemic storm (Mudd et al., 2020; Wang et al., 2020a). Based on this premise, data mining of the literature for ARDS studies in which measures of cytokine levels in BALF and blood are provided in the same patients yielded the results presented in Tables 1 and 2.

Table 1 presents data from ARDS of pulmonary origin, while Table 2 presents data from extrapulmonary causes. Of note, none of the studies specifically addressed a potential lung-to-systemic cytokine gradient. Further, the sample sizes are rather small, particularly for the two COVID-19 cases in Table 1. Finally, no attempt is made to establish statistical differences. Despite these limitations, there is a notable trend for higher levels of inflammatory cytokines in BALF than plasma of ARDS patients in which the inciting event was of pulmonary origin (Table 1). This lung-to-systemic gradient is not as evident in ARDS of non-pulmonary origin (Table 2). Analogous results are obtained in experimental models of lung inflammation and injury. When the inciting factor is a direct insult to the lungs, the BALF levels of cytokines exceed their blood levels (Table 3). Of note, dismantling of NETs within the airspace (intratracheal DNase), reduces LPS-induced alveolar damage. This maneuver decreases the cytokine levels in both compartments, while maintaining a BALF-to-lung cytokine gradient (Table 3). By contrast, when lung inflammation/injury is a result of an indirect insult (e.g., peritonitis), a BALF-to-blood cytokine gradient is not evident (Table 4). Collectively, a BALF-to-blood cytokine gradient is noted in ARDS and animal models when the inciting event is of lung origin. Thus, despite the limited data from COVID-19 cases, it seems likely that a lung-centric cytokine storm may characterize the ARDS of COVID-19.

**TABLE 1 | Systemic and bronchoalveolar lavage fluid (BALF) cytokines in ARDS of pulmonary origin.**

|        | Systemic | BALF   | Time  | n  | Study |
|--------|----------|--------|-------|----|-------|
| **MCP1** |          |        |       |    |       |
| Pneumonia | 80       | 3,000  | 3 hrs | 5  | 1     |
| COVID    | 1,660    | 3,470  | 3 days| 4  | 2     |
| **IL-8** |          |        |       |    |       |
| Pneumonia | 60       | 300    | 3 days| 47 | 3     |
| Pneumonia | 370      | 463    | 1 day | 44 | 4     |
| Pneumonia | 30       | 761    | 3 hrs | 5  | 1     |
| COVID    | 1,250    | 3,375  | 3 days| 4  | 2     |
| **IL-1β** |         |        |       |    |       |
| Pneumonia | 51       | 95     | 1 day | 44 | 4     |
| Pneumonia | 7        | 100    | 3 hrs | 5  | 1     |
| **TNF-α** |          |        |       |    |       |
| Pneumonia | 15       | 77     | 1 day | 44 | 4     |
| Pneumonia | 15       | 300    | 3 hrs | 5  | 1     |
| Pneumonia | 30       | ND*    | 3 days| 47 | 3     |
| COVID    | ND       | ND     | 3 days| 4  | 2     |
| **IL-6** |          |        |       |    |       |
| Pneumonia | 1,500    | 3,000  | 3 days| 49 | 3     |
| Pneumonia | 220      | 283    | 1 day | 44 | 4     |
| Pneumonia | 317      | 919    | 3 hrs | 5  | 1     |
| COVID    | 896      | 3,786**| 21-23 days | 1 | 5     |
| COVID    | 1,560    | 1,300  | 3 days| 4  | 2     |

Values are the means in pg/ml. The means were either given or approximated from measurements provided (transparent grid overlay). Time: time of sample collections in hours/days after start of mechanical ventilation. *ND, not detected; **IL-6 levels in pleural effusion = 18,000 pg/ml. Study 1 (Osaki et al., 2010): ARDS, bilateral infiltrates, 1/5 patients died. Study 2 (Florit et al., 2021): ARDS, bilateral infiltrates, lymphopenia, 2/4 patients died. Study 3 (Scharte et al., 1996): ARDS, microorganisms detected in BALF, 42% of patients died. When BALF levels of IL-8 and IL-6 were corrected for urea, the predicted levels in alveolar fluid were 10-fold higher, indicating local production of the two cytokines. Study 4 (Lee et al., 2010): acute respiratory failure due to severe pneumonia, 34/44 patients died. BALF cytokine levels exceeded systemic levels regardless of the comparisons made (e.g., survivors vs non-survivors). Study 5 (Wang et al., 2020a): ARDS, septic shock, multiple organ failure, the patient died. Based on the IL-6 gradient from lungs to blood, proposed that “the local (cytokine) storm may be worse than the systemic storm”. Compartments with higher levels in bold font.
TABLE 2 | Systemic and bronchoalveolar lavage fluid (BALF) cytokines in ARDS of non-pulmonary origin.

| Cytokine | Systemic | BALF | Time | n | Study |
|----------|----------|------|------|---|-------|
| IL-8     | 3,525    | 480  | 2 hrs| 20| 1     |
|          | 630      | 3540 | 17 days| 6 | 2     |
|          | 20       | 250  | 3 days| 10| 3     |
| IL-1β    | 460      | 11,900| 17 days| 6 | 2     |
|          | 364      | 267  | 2 hrs| 23| 4     |
| TNF-α    | 370      | 3,900| 17 days| 6 | 2     |
|          | 204      | 463  | 2 hrs| 23| 4     |
| IL-6     | 40       | ND*  | 3 days| 10| 3     |
|          | 388      | 538  | 2 hrs| 20| 1     |
|          | 880      | 9,780| 17 days| 6 | 2     |
|          | 400      | 4,000| 3 days| 10| 3     |

Values are the means in pg/ml, except study 4 in which values are the medians in pg/ml. The values were either given or approximated from measurements provided (transparent grid overlay). Time: time of sample collections in hours/days after start of mechanical ventilation. *ND, not detected. **Samples from distal small bronchi, presumed to represent alveolar fluid. Adverse reaction: ARDS: respiratory failure, bilateral infiltrates; diagnosis: trauma (9), pneumonia (3), sepsis (2), pancreatitis (2), urosepsis (2), shock (2), burns (1); 14/20 patients died. Regardless of comparisons made (e.g., survivors vs non-survivors) IL-6 levels in BALF exceeded systemic levels, while systemic levels of IL-8 exceeded their BALF levels. Study 2 (Meduri et al., 1995): ARDS, respiratory failure, lung PMN infiltrates; diagnosis: pneumonia (3), aspiration (1), urosepsis (3), intra-abdominal infection; (1) 4/8 patients died. Time of sampling varied from 5 to 30 days after ARDS with a mean of 17 days. Study 3 (Schutte et al., 1996): ARDS, respiratory failure, lung PMN infiltrates; diagnosis: sepsis (non-pulmonary origin) (7), shock (3); 6/10 patients died. Study 4 (Agouridakis et al., 2003): ARDS, respiratory failure, bilateral infiltrates; diagnosis: trauma (9), pneumonia (5), sepsis (2), necrotizing pneumonia (2), intubation (2), intubation (1), burns (1); 12/23 patients died. Compartment with higher levels in bold font.

Inherent in experimental models of lung inflammation/injury is a defined time interval from insult to assessment of endpoints. Chemokines (e.g., IL-8, MIP-2) are detected as early as 2 – 6 hours after direct injury to the lungs, exceeding plasma levels by at least 20-fold (Table 3). Further, the acid-induced lung injury (pulmonary edema and impaired oxygenation) is associated with the presence of neutrophils in the BALF (Folkesson et al., 1995). Both neutrophil recruitment and lung injury are prevented by chemokine (post-acid) blockade of IL-8. These observations are consistent with the following scenario. Acid stimulates lung epithelium and/or macrophages to generate IL-8, which attracts neutrophils to the lungs, where they are activated and cause injury (Folkesson et al., 1995).

There are a few issues of relevance to COVID-19 that warrant attention. When BALF and plasma samples are obtained early after admission to ICU, a lung-to-blood gradient for the chemokines, IL-8 is detected (Table 1); correspondingly, the BALF also contains activated monocytes and neutrophils (Ronit et al., 2021). In the same samples, no such gradient was detected for the pro-inflammatory cytokines, TNF-α or IL-6 (Ronit et al., 2021). However, in a longitudinal study of one patient with protracted COVID-19 (over 3 weeks), a 4-fold BALF to blood gradient for IL-6 was attained just days before death (Table 1). Of note, in pleural effusion samples obtained concurrently, the level of IL-6 was 20-fold greater than in plasma (Wang et al., 2020a). Further, assuming a progression in disease severity over...

TABLE 3 | Systemic and bronchoalveolar lavage fluid (BALF) cytokines in animal models of direct lung injury.

| Cytokine | Systemic | BALF | Time  | n  | Study |
|----------|----------|------|-------|---|-------|
| IL-8     | 330      | 6,700**| 6 hrs| 10| 1     |
| Acid     |          |       |       |    |       |
| MIP-2    | ND*      | 200  | 2 hrs| 4 | 2     |
| VILI     |          |      |       |    |       |
| IL-1β    | ND       | 30   | 2 hrs| 4 | 2     |
| VILI     |          |      |       |    |       |
| IL-6     | 2,125    | 3,050 | 24 hrs| 16| 3     |
| LPS      | 270      | 1,670| 24 hrs| 6 | 3     |
| TNF-α    | ND       | ND** | 2 hrs| 4 | 2     |
| VILI     |          |      |       |    |       |
| LPS      | 450      | 1,460| 24 hrs| 6 | 3     |
| DNase/LPS| 280      | 500  | 24 hrs| 6 | 3     |

Values are the means in pg/ml. The values were either given or approximated from measurements provided (transparent grid overlay). Time: time of sample collections in hours after direct insult to lungs. *ND, not detected. **Samples from distal small bronchi, presumed to represent alveolar fluid, contained 40,500 pg/ml IL-8. Study 1 (Folkesson et al., 1995): Hydrochloric acid given intratracheally to rabbits. Indices of lung injury: lung edema and systemic hypoxia. PMN infiltration. All rabbits died within 12 - 14 hours after lung injury. Study 2 (Ricard et al., 2001): ventilator-induced lung injury in rats (VILI 42 ml/kg tidal volume). Index of lung injury: Increased protein levels in BALF. Study 3 (Liu et al., 2016): lipopolysaccharide (LPS) given intratracheally to mice. Indices of lung injury: interstitial edema, PMN infiltration, hemorrhage, NET components in BALF and lung tissue. Intratracheal DNase reduced NET formation and lung injury. Compartment with higher levels in bold font.
time, additional insight is gained by comparisons of moderate to severe cases of COVID-19. Chemokines (e.g., IL-8) dominate the BALF profile in moderate cases, whereas pro-inflammatory cytokines (e.g., TNF-α, IL-6) are prevalent in more severe cases (Liao et al., 2020). The BALF of moderate COVID-19 patients is enriched with macrophages, while BALF of severe cases is enriched with neutrophils, with the neutrophil count being directly related to the levels of IL-8 (Pandolfi et al., 2020). Thus, the SARS-CoV-2 infection of the lungs appears to follow the expected trajectory of an inflammatory response (Folkesson et al., 1995).

**LUNG-CENTRIC IMMUNOTHROMBOSIS**

The recruitment and activation of neutrophils can result in the formation of neutrophil extracellular traps, or NETs (Figure 1). NETs are an extruded web of decondensed chromatin DNA decorated with granule-derived proteases and antimicrobials (Papayannopoulos, 2018; Boeltz et al., 2019). The formation of NETs are an extruded web of decondensed chromatin DNA (Middleton et al., 2020; Radermecker et al., 2020; Veras et al., 2018). In lung tissues of fatal COVID-19 cases, NETs have NETs to the extracellular space (Tall and Westerterp, 2019; Chen et al., 1995).

**TABLE 4 | Systemic and bronchoalveolar lavage fluid (BALF) cytokines in animal models of indirect lung injury.**

| Study | Systemic | BALF | Time | n | Study |
|-------|----------|------|------|---|-------|
| DH    | 600      | 25   | 12hrs| 7 | 1      |
| KC    | 1500     | 100  | 12hrs| 7 | 1      |
| IL-6  | 20,000   | 20,000| 24 hrs| 6 | 3      |
| CLP   | 9,000    | 5,000| 6 hrs| 6 | 2      |
| IL-1β | 90       | 180  | 24 hrs| 6 | 3      |
| CLP   | 50       | 60   | 6 hrs| 6 | 2      |
| TNF-α | 280      | 220  | 24 hrs| 6 | 3      |
| CLP   | 50       | 25   | 6 hrs| 6 | 2      |

Values are means in pg/ml. The values were either given or approximated from measurements provided (transparent grid overlay). Time: time of sample collections in hours after insult. Study 1 (Kalbitz et al., 2019): mice were subjected to a double-hit (DH) insult consisting of bilateral lung contusion followed 24 hrs later by cecal ligation and perforation. Indices of lung injury: protein in BALF, MPO activity in lung homogenates. Study 2 (Wang et al., 2019): mice were subjected to cecal ligation and perforation (CLP). Indices of lung injury: inflammatory cell infiltration, alveolar damage, and edema in lung tissue. CLP also injured the heart, liver, and kidneys. 9/20 mice died by 24 hrs after CLP and 15/20 died by 7 days. Study 3 (Wang et al., 2020): mice were subjected to cecal ligation and perforation (CLP). Indices of lung injury: inflammatory cell infiltration, alveolar damage, and edema in lung tissue. Compartment with high levels in bold font.

thrombogenesis in a cohort of acute coronary syndrome patients with high circulating CRP (Liberale et al., 2019). The lungs are particularly susceptible to immunothrombosis, given the readily available pool of neutrophils (Granton et al., 2018) and platelets (Lefrancçois et al., 2017). In this scenario, a viral-induced inflammation promotes the formation of NETs by activated neutrophils. The NETs serve as a scaffold for sequestering activated platelets and components of the coagulation cascade (Mcdonald et al., 2017), setting the stage for the generation of thrombi. Occlusive thrombi within the pulmonary vasculature have been noted in fatal COVID-19 cases (Ackermann et al., 2020; Leppkes et al., 2020; Radermecker et al., 2020; Rapkiewicz et al., 2020). Of note, anticoagulants (e.g., heparinoids) are advocated to alleviate the hypercoagulation state of COVID-19 (Bikdeli et al., 2020; Connors and Levy, 2020; Leentjens et al., 2021). However, given the potential for bleeding, specific guidelines for thromboprophylaxis in these patients await the outcome of ongoing clinical trials (Leentjens et al., 2021).

Circulating DNases can degrade the DNA backbone of NETs and may serve as an endogenous regulatory mechanism to limit immunothrombosis (Jiménez-Alcázar et al., 2017; Mcdonald et al., 2017). In murine models of sepsis, dismantling of NETs by DNases reduces occlusive intravascular clots in the lungs and improves survival (Jiménez-Alcázar et al., 2017; Lefrancçois et al., 2018). In ARDS patients (all causes), the severity of disease and lethality is related to the ratio of plasma NETs/DNases (Lefrancçois et al., 2018). A similar situation appears to exist in COVID-19 patients, circulating NETs are increased with a corresponding decrease in DNase levels (Lee et al., 2021).

In fatal cases of COVID-19 microvascular thrombi and necrotic injury in organs remote from the lungs have been noted on autopsies (Ackermann et al., 2020; Rapkiewicz et al., 2020; Tang et al., 2020). Potential mechanisms include spill-over of either the virus or host cytokines from damaged lungs into the systemic circulation. However, there is little evidence that viable SARS-CoV-2 becomes blood-borne (Andersson et al., 2020; Lamouroux et al., 2020).
2020; Vivanti et al., 2020). Further, circulating levels of proinflammatory cytokines do not reach levels considered detrimental to tissues (Kox et al., 2020; Mudd et al., 2020; Sinha et al., 2020). Whether the pulmonary NET-mediated immunothrombosis of COVID-19 can impact remote organs is not clear at present. While NETs and NET-associated thrombi have consistently been found in the lungs of fatal cases, their presence in remote organs is equivocal (Leppkes et al., 2020; Radermecker et al., 2020). Alternatively, while NET remnants (presumably due to DNase-induced turnover) occurs in COVID-19 (Leppkes et al., 2020), the initiated hypercoagulative and thrombotic milieu may be a source of NET-independent remote organ involvement (Connors and Levy, 2020). Given the paucity of information on this issue, any conclusions regarding mechanisms of remote organ injury in COVID-19 are rather speculative.

**POTENTIAL LUNG-CENTRIC THERAPY**

The lung-centric inflammatory response prompts consideration of the potential clinical utility of bronchoscopy and BALF. BALF analyses are currently used to provide a microbiological diagnosis of COVID-19 in suspected cases, but in which nasopharyngeal swabs are negative for viral RNA (Mondoni et al., 2020; Patrucco et al., 2020). In addition, BALF analyses can also direct specific antimicrobial therapy and bronchoscopy can be used to clear the bronchial passage (Bruyneel et al., 2020). Of interest from a therapeutic perspective is the results of a murine study in which local lung inflammation and injury was induced by intratracheal LPS (Table 3). The recruited and activated PMN generated NETs within the airspace, as evidenced by NET markers in BALF (Liu et al., 2016). Unlike NETs formed in blood vessels, which are rapidly cleared (in part via DNase), NETs in the airspace are stable structures (Lefrançois et al., 2018). Exogenous intratracheal DNase reduces NETs in the BALF with a corresponding reduction in both BALF and systemic cytokines (Table 3). Of relevance to the ARDS of COVID-19, hypoxemic patients on ventilators were given nebulized recombinant human DNase I as part of their therapeutic regimen; no adverse effects were noted and the potential for benefit has prompted several clinical trials (Weber et al., 2020).

The possibility of directly targeting cytokines generated within the lungs of COVID-19 patients is attractive for at least two reasons. First, based on the lung-centric cytokine storm, reducing lung cytokines or their activity would also reduce corresponding circulating levels and their effects on remote organs. Second, intrapulmonary therapeutics would be less prone to off-target systemic effects. Ideally, an initial assessment of the BALF levels of cytokines in COVID-19 patients should be made as soon as possible after diagnosis. This would allow for therapeutic targeting of relevant cytokines. While early intervention would maximize patient benefit, limiting lung inflammation even in advanced cases is desirable.

The rapidly expanding repertoire of animal models of COVID-19 (Cleary et al., 2020; Muñoz-Fontela et al., 2020) should facilitate the translation of experimental findings to the clinical realm. While many of the models lack specific features noted in the human disease (e.g., lethality) (Ehaideb et al., 2020), some do have notable similarities to severe COVID-19. In this regard, transgenic mice expressing ACE2 under the cytokeratin 18 promoter (K18-hACE2) hold promise (Winkler et al., 2020; Yinda et al., 2021). These mice exhibit severe lung inflammation and dysfunction, lymphopenia, evidence of coagulopathy, remote organ involvement, and fatality. As it appears to be the case in COVID-19, viral RNA can be detected in remote organs of K18-hACE2 mice despite the absence of viremia (Yinda et al., 2021). Since murine models are readily amenable to experimental manipulation, this enigma may be resolved in future studies. Further, experiments to more directly address lung-centric immunothrombosis (e.g., NET formation, BALF to blood cytokine gradient) in these and other genetically tractable mice (Jarnagin et al., 2021) should provide a basis for lung-centric directed therapy.

**AUTHOR CONTRIBUTIONS**

Conceptualization: PK and HF. Literature search: PK, HF, and SK. Data analysis and interpretation: PK and HF. Drafting the article: PK and HF. Critical revision of the article: PK, HF, SK, AY, EA-M, and KA-K. All authors contributed to the article and approved the submitted version.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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