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The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease

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\textbf{A B S T R A C T}

Severe COVID-19 associated pneumonia patients may exhibit features of systemic hyper-inflammation designated under the umbrella term of macrophage activation syndrome (MAS) or cytokine storm, also known as secondary haemophagocytic lymphohistocytosis (sHLH). This is distinct from HLH associated with immunodeficiency states termed primary HLH -with radically different therapy strategies in both situations. COVID-19 infection with MAS typically occurs in subjects with adult respiratory distress syndrome (ARDS) and historically, non-survival in ARDS was linked to sustained IL-6 and IL-1 elevation. We provide a model for the classification of MAS to stratify the MAS-like presentation in COVID-19 pneumonia and explore the complexities of discerning ARDS from MAS. We discuss the potential impact of timing of anti-cytokine therapy on viral clearance and the impact of such therapy on intra-pulmonary macrophage activation and emergent pulmonary vascular disease.

1. Introduction

We live in extraordinary times with intensive care units around the globe being overwhelmed with severe COVID-19 viral pneumonia that leads to severe adult respiratory distress syndrome (ARDS). Given the lack of a vaccine or proven effective anti-viral therapy and non-existent herd immunity, anti-cytokine therapy, most notably anti-IL-6 and others including IL-1 antagonism have been proposed for mitigating hyper-inflammation that may develop in conjunction with this virally induced ARDS \cite{1,2}. The backdrop for cytokine antagonism of hyper-inflammation in non-immunodeficient patients emerged in children with systemic onset juvenile inflammatory arthritis (sJIA) also known as Stills disease where a severe hyper-cytokineemic inflammatory state variously termed as a cytokine storm, macrophage activation syndrome (MAS) or secondary haemophagocytic lymphohistocytosis (sHLH) may occur \cite{3-5}. Impressive responses to antagonism of either IL-6 or IL-1 have been reported in sJIA \cite{6,7}.

The suspicion that overzealous immune responses associated with MAS/sHLH may be driving COVID-19 related ARDS has created a tremendous interest for anti-cytokine therapy for dampening of such exaggerated immune responses as a beneficial therapeutic strategy \cite{2}. The focus of this article is to explore COVID-19 pulmonary immunopathology and describe the potential benefits or disadvantages of IL-6 antagonism is patients with severe inflammatory responses that has implications for other anti-cytokine strategies including IL-1, IL-18 or IFN\gamma antagonism. Other DMARDs may also have potential use for treating COVID-19 patients, and excellent reviews can be found elsewhere \cite{8}.

2. Macrophage activation syndrome in COVID-19 pneumonia?

Although pneumonitis may occur, the classic MAS/sHLH picture in sJIA and other settings most often occurs outside the lungs and manifests as fevers, adenopathy, hepatosplenomegaly, anaemia, other cytopenias, liver function derangement and the activation of intravascular coagulation cascade secondary to inflammation, and is accompanied by marked hypercytokinaemia. (Fig. 1A) Clinical and laboratory parameters in the MAS/sHLH phenotype are similar to primary HLH but the latter is invariably autosomal recessive, presenting in childhood, and is typically due to mutations that impair NK and CD8+ cytotoxic T-cell function \cite{9-11}, although there are emergent overlaps. As the focus of this perspective is restricted to IL-6 in potential MAS in COVID-19 related pneumonia, the reader is referred to several recent articles on primary and secondary HLH and sJIA with MAS \cite{12-15}.

Laboratory parameters including highly elevated CRP and hyperferritinaemia, the latter of which may play a complex role in disease \cite{16-18}, are key to the diagnosis of MAS/HLH and are elevated in many severe COVID-19 pneumonia cases. Other features including coagulopathy and abnormal liver function may be evident suggesting that a subgroup of COVID-19 pneumonia cases also have MAS/sHLH.
The limited COVID-19 post mortem data shows prominent alveolar oedema, hyalnosis (intra-alveolar proteinosis) and fibrin deposition with pneumocytes viral cytopathic change and immune cell infiltration including lymphocytes is typical or ARDS [26] as is evolving severe multi-organ damage including renal, cardiac and liver dysfunction with hypoproteinaemia [28]. In patients with ARDS (not generally due to viral pneumonia, but other causes) the elevation in baseline plasma levels of IL-6 predicted a poor survival [29] as did even higher bronchoalveolar (BAL) fluid levels indicating a pulmonary, rather than systemic origin for these cytokines in ARDS pathology [30]. Therefore, the overlapping cytokine profiles between severe ARDS and MAS/shHLH may limit the utility of cytokine profiling in the differentiation between both conditions and many of the laboratory changes reported in COVID-19 could predominantly reflect ARDS. (Fig. 2).

3. A new proposed integrated innate and adaptive immune mechanisms in MAS/shHLH

The link between a COVID-19 induction of a temporary immunodeficiency states (with features resembling primary HLH) rather than MAS/shHLH (that may occur in completely immunocompetent states) is not something yet that is fully appreciated. At first glance, the notion of hyper-inflammation in the face of immunodeficiency seems counter-intuitive, but that is exactly what happens in primary HLH. Inflammation against self occurs along an immunological disease continuum with pure innate and pure adaptive immunity at opposite
boundaries [31]. Within these clear boundaries it is possible to stratify immune responses as being loss of function, normal or gain of function which then permits a better conceptual understanding of the integrated workings of the system [32] (Fig. 3).

Primary HLH broadly defines hyper inflammatory immunodeficiency states that often equate with failure of the perforin and NK and CD8+ cytotoxic T-cells killing machinery that forms pores leading to cell lysis to initiate apoptosis of virally infected cells [9,33,34]. This leads to widespread T-cell mediated IFN-γ driven secondary cytokine driven macrophage activation (Fig. 3A). Vigorous immunosuppression and sometimes anti-viral chemotherapy in these settings only represents a bridging strategy towards definitive allo-geneic bone marrow transplantation strategies with immunosuppression being futile. Indeed evidence for such a primary HLH with immunodeficiency picture in adults succumbing to H1N1 influenza viral pneumonia has been reported [19], making it possible that patients succumbing to COVID-19 may also occasionally harbour perforin pathway mutations.

The COVID-19 associated pneumonia is associated with lung damage and ARDS and robust interferon suppression with lymphopenia as part of the virally induced immunosuppression. Also, preliminary data suggest that disease severity in COVID-19 may be associated with low IFN-γ production by CD4 + T-cells [35]. The related SARS-CoV virus open reading frame (ORF) and N proteins, act as antagonists to the interferon pathway by regulating IFN-β synthesis and signalling [36] which was mirrored in another experimental model [37]. In MERS-CoV-infected rhesus macaques, treatment with interferon-α2b was able to improve outcome [38]. Both IFN-β and IFN-γ inhibit the replication of SARS-CoV [39]. A characteristic feature of primary HLH but not sHLH/MAS is defective NK function which is also reported in COVID-19 infection, but by different mechanisms [40].

Analogous to primary HLH, the loss of “front line” anti-viral defence mechanism may activate a “second wave” of more tissue aggressive immunity including exaggerated IL-6 production with a secondary cytokine storm supervening with increased tissue damage (Fig. 3C) and (Fig. 4). Other “second wave” of non-type-1 interferon pathway myeloid and stromal derived cytokines including IL-1, TNF, IL-18, GM-CSF would be expected to be part of blood hyper-cytokinaemia and MAS picture (Panel 4C). Accordingly, there are similarities between COVID-19 and primary HLH which would point towards the importance of viral load reduction in COVID-19 (Fig. 3C).

It is also postulated that the typical MAS/sHLH pathology that arises in immunocompetent cases (Fig. 3B) may also arise in the COVID-19 infection (Fig. 3D). However, how this occurs in the face of active infection and how these two patterns of MAS could be differentiated and whether this clinically matters, especially in advanced ARDS is presently unclear. Stated differently, such a hypersensitive T-cell mediated reaction against virally infected cells would be expected to clear the actual infection, but contributing to ongoing damage and ARDS (Fig. 4D). Viraemia has been reported in up to 40% of cases [41] with one study showing a strong correlation between serum viral RNA load and ARDS severity [42]. A simple serological score including serial measurements of CRP, ferritin and blood viral load could therefore be used to evaluate therapy strategies for these different types of MAS (Table 2), but it is essential to appreciate that the hypoxaemic environment of ARDS complicates the perceived MAS picture.

4. Interleukin-6 and immune function in COVID-19 related pneumonia

Clues as to how the increased levels of IL-6 and other cytokines that arise in ARDS impact on immunity come from experimentally induced viral lung infection where IL-6 may have contextual protective or exacerbating roles including severity of infection, survival and tissue remodeling, but there are very limited data on coronavirus family members in general (Table 1). Interleukin-6 also plays an important role in lung repair responses following viral or chemical insults indicating that timing of administration of anti-IL6R could impact on proper tissue remodeling (Table 1). In human epithelial cells, SARS-CoV was able to induce greater IL-6 when compared to Influenza-A virus and human influenza virus type 2, but interestingly induced less SOC3 than other viruses, suggesting a potential basis for exaggerated IL-6 responses with this family of viruses [43].

A picture emerges of COVID-19 ARDS and “second wave” pro-inflammatory cytokines including IL-6 and others leading to the MAS like pathology (Fig. 4C and D). The biology of IL-6 is complex with cytokine engagement of membrane anchored IL-6R and gp130 co-receptor being known to have tissue homeostatic and repair responses [44]. However, many non-immune cells including stromal and epithelial cells can induce marked inflammatory responses when soluble IL-6R/IL-6 anchors to membrane gp130 in what is termed trans-signalling. This engagement potently activates inflammatory responses [45] (Fig. 4B). Reports indicate that murine pulmonary stromal cells including myofibroblasts signal via both IL-6R and trans signalling but type 2 pneumocytes lack the soluble membrane IL-6 receptor indicating that these signals exclusively via IL-6R trans signalling [46] (Fig. 4D). Given that trans signalling typically drives inflammatory reactions this may impact on COVID-19 immunity [47-49].

5. Key messages and considerations for IL-6 for COVID-19 pneumonia, ARDS and MAS

We highlight how COVID-19 pneumonia may represent a novel viral MAS-like immunopathology, where hyper-inflammation may be key to virus control in the face of disabled type-1 interferon responses. Furthermore, the recognition of MAS/sHLH is problematic in COVID-19 pneumonia cases with the severe inflammation emanating from the pulmonary compartment mimicking MAS, but the lack of other classical systemic clinical features making MAS presentation atypical and diagnosis more difficult. Consequently, many cases receiving anti-IL6R or other cytokine inhibitors therapy may have severe infection related ARDS without superimposed MAS. Although inflammation is more lung centered, than multi-organ, the argument for IL-6 involvement in COVID-19 related MAS comes from biochemical parameters changes including ferritin and the preliminary open reports of anti-IL6R efficacy [28,50]. We appear to be dealing with a pulmonary pathology distinct from MAS with DIC with both the macrophage activation and
associated coagulopathy being more centered on the lung and not systemic (Figure 1B).

Considerations around timing of anti-IL-6 therapy and its use outside a clear-cut systemic MAS pictures are key. Early use of anti-retroviral therapy strategies to reduce viral load appear crucial to preventing the relative immunosuppression that might be contributing to the MAS like picture development. The subtle overlap in features of severe COVID-19 pneumonia with primary HLH and the sub-analysis of the failed anti-retroviral trial in COVID-19 pneumonia suggested a potential benefit of early anti-viral therapy initiation [1,51].

It is presently unclear if elevated IL-6 levels are detrimental or beneficial in COVID-19 pneumonia. In experimental model systems, IL-6 can either suppress or facilitate viral replication [49], so studies on COVID-9 are urgently needed. Timing of anti-IL-6R, if too early might adversely affect viral clearance which needs to be assessed in trials. If it emerges that blocking IL-6R early in the course of COVID pneumonia MAS-like disease has a detrimental impact on type-2 pneumocyte antiviral immunity, then local augmentation of IL-6 could be considered. Such are the complexities that only trial results will clarify.

In this perspective we focused on IL-6 and its relationship to the COVID-19 MAS-like pathology but several other relevant cytokines including IL-18, IFNγ, and the JAK1 pathway critically control
macrophage function including IL-6 production during MAS states [52]. Antagonism of either IFN-γ or IL-18 that may be upstream of IL-6 and IL-1 has been associated with efficacy in human HLH and SJIA [53,54]. Both the short term and long-term outcome of trials of IL-6 blockers in COVID-19 pneumonia are eagerly awaited to clarify nature of the MAS-like state.

If a MAS-like state exists and excessive IL-6 levels are detrimental—why shouldn’t corticosteroids be first line therapy as these will vigorously suppress IL-6 and a raft of other cytokines? Although the recent open label study from Wu and colleagues showed a benefit for corticosteroids, the consensus is that these should not be used based on clinical experience in SARS-CoV, MER-CoV and other infections including influenza and respiratory syncytial virus infection, where collectively there is evidence for delayed viral clearance [20,55]. The MAS-like state in COVID-19 exhibits features of both primary and secondary HLH with death being linked to respiratory viral persistence.

**Table 1**

Impact of Interleukin-6 on viral pneumonitis and lung Injury.

<table>
<thead>
<tr>
<th>Experimental model</th>
<th>Key findings</th>
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<tr>
<td>Infection of human epithelial cells with SARS-CoV</td>
<td>SARS-CoV induces greater IL-6 when compared to Influenza-A virus and human parainfluenza virus type 2</td>
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<td>Infection of monocytes/macrophages with SARS-spike protein</td>
<td>Disease dependent on infiltrating monocytes, which produced high levels of IL-6, IL-1β and TNF</td>
<td>[60,61]</td>
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<td>Murine SARS-CoV model</td>
<td>IL-6 KO mice show increased mortality</td>
<td>[62]</td>
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<td>Influenza infection with IL-6 KO mice</td>
<td>IL-6 KO mice show increased macrophage phagocytic function, fibroblast proliferation, migration and collagen deposition, prevents viral induced neutrophil death</td>
<td>[63,64]</td>
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<tr>
<td>HSV-1 respiratory infection with IL-6 KO mice</td>
<td>IL-6 KO mice show increased macrophage phagocytic function, fibroblast proliferation, migration and collagen deposition, prevents viral induced neutrophil death</td>
<td>[65]</td>
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<tr>
<td>Experimental ARDS induced by the intratracheal instillation of bacterial endotoxin</td>
<td>Reduced pulmonary inflammation by the addition of recombinant IL-6</td>
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<td>Infectious models of tuberculosis, pneumococcal pneumonia and pulmonary aspergillosis</td>
<td>IL-6 KO mice show increased mortality in all infectious models</td>
<td>[68,70]</td>
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<tr>
<td>Bleomycin lung injury model with IL-6 neutralization</td>
<td>IL-6 neutralization resulted in accelerated type 2 pneumocyte apoptosis, neutrophilic inflammation, accelerated lung fibrosis</td>
<td>[71]</td>
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<tr>
<td>Rat model of bacterial sepsis remote from the lung with tocilizumab</td>
<td>Tocilizumab (anti-IL-6) resulted in reduced sepsis-induced pulmonary and renal inflammatory, decreased mortality</td>
<td>[72]</td>
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in the aforementioned Wu et al. study, indicating that, analogous to primary HLH, ongoing infection may be a driver. The role of IL-6 and other cytokines in what could be a distinct MAS-like lung inflammation with associated inflammation driven pulmonary vascular disease awaits clarification.

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References


