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REVIEW ARTICLE

The pathogenic role of epithelial and endothelial cells in early-phase COVID-19 pneumonia: victims and partners in crime

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Abstract

Current understanding of the complex pathogenesis of COVID-19 interstitial pneumonia pathogenesis in the light of biopsies carried out in early/moderate phase and histology data obtained at postmortem analysis is discussed. In autopsies the most observed pattern is diffuse alveolar damage with alveolar-epithelial type-II cell hyperplasia, hyaline membranes, and frequent thromboembolic disease. However, these observations cannot explain some clinical, radiological and physiopathological features observed in SARS-CoV-2 interstitial pneumonia, including the occurrence of vascular enlargement on CT and preserved lung compliance in subjects even presenting with or developing respiratory failure. Histological investigation on early-phase pneumonia on perioperative samples and lung biopsies revealed peculiar morphological and morpho-phenotypical changes including hyper-expression of phosphorylated STAT3 and immune checkpoint molecules (PD-L1 and IDO) in alveolar-epithelial and endothelial cells. These features might explain in part these discrepancies.

Introduction

Coronavirus disease 2019 (COVID-19) is a pandemic disease characterized by clinical variability, with patients developing mild symptoms and others experiencing an interstitial pneumonia that can rapidly progress, in a minority of cases, to severe life threatening respiratory failure requiring mechanical ventilation or even extra corporeal membrane oxygenation ECMO [1]. The worldwide spread of infection and the lack of reliable and accepted therapies to prevent and cure the severe form and its possible consequences urge for a clear understanding of the pathogenic mechanisms and an explanation for the observed clinical variability. Although it is now widely accepted that infection can trigger a hyper-inflammatory response (also termed "cytokine storm") [2], the pathogenic role of different cell types and the mechanisms leading to different disease phases and endotypes are not fully understood [3]. The heterogeneity of clinical presentations is likely conditioned by the viral burden, the efficacy of innate and adaptive immune responses, genetic predisposition, and the occurrence and severity of preexisting comorbidities (such as older age, obesity, hypertension, and diabetes mellitus) [4, 5]. From the beginning of COVID-19 pandemic, when soon the need of pathological data became evident, a large

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number of postmortem studies have been performed worldwide, providing relevant information mainly regarding the pathogenic mechanisms occurring in severe cases (extended lists of references are available in previous reviews) [3, 6–10].

Postmortem studies

All reports indicate the lungs as the principal target of SARS-CoV-2 in deceased cases [3, 6–14]. The most frequent pathological pattern observed in autoptic lung is "diffuse alveolar damage" (DAD), with hyaline membranes, alveolar fibrinous edema and type-II alveolar-epithelial cell (AECII) hyperplasia. Most cases present features typical of the exudative phase, whereas the proliferative findings including interstitial fibrosis are common in subjects with a long lasting clinical history. Such aspects occur in acute respiratory distress syndrome (ARDS) regardless of the cause, including H1N1 pneumonia, SARS, and MERS, so that the "peculiarity" of the pathogenic mechanisms triggered by the SARS-CoV-2 has been challenged [15, 16]. In postmortem reports the time lapse from symptoms onset to tissue sampling was highly variable, and pulmonary superinfections were also documented in a significant number of cases. Inflammatory cell accrual was generally described as heterogeneous, with macrophage accumulation in most cases, variable lymphocyte infiltration (sometimes perivascular) and occasional widespread infiltration of granulocytes mainly when a superimposed bacterial infection was documented. In many studies the occurrence of thrombotic events was reported, varying from small capillary clots to thrombosis in the larger vessels [17–20]. These latter findings have been linked up to the higher frequency of coagulation abnormalities observed in COVID-19, compared to those with non-COVID-19 ARDS, and were considered clinically relevant, advocating the need for anticoagulation therapeutics [21, 22]. Infiltration of vascular wall by inflammatory cells with necrosis (vasculitis) was also rarely reported.

The general view that the DAD pattern can be sufficient to explain the pathogenesis of COVID-19 progression may be disputed for several reasons. First, the possible pathogenic contribution of confounding factors, including the use of oxygen at high doses and the impact of barotrauma due to the positive pressure mechanical ventilation should be considered. The complications following intubation, such as secondary ventilator-induced lung injury are well known and could be the cause of some DAD features reported at autopsy [23]. The impact of pulmonary thromboembolism or in situ thrombosis and superimposed inflammation due to bacterial and/or fungal infections should be also taken into account. Secondly, in a minority of reports different patterns are described, including organizing pneumonia, acute fibrinous organizing pneumonia and lymphocytic viral pneumonia [24, 25]; this heterogeneity may suggest to avoid a simplistic histological lumping [26]. Significant heterogeneity has also been revealed in COVID-19 by immunological, radiological, and clinical studies, accounting for the evidence of different disease phenotypes [27–29]. In fact, a number of studies have revealed that COVID-19 may present with features that are not typical for ARDS, including a preserved compliance, hyper-perfusion of affected pulmonary areas, loss of hypoxic vasoconstriction, and "silent hypoxia" (or "happy hypoxia"), i.e., a state of low oxygen saturation without subjective perception of respiratory distress [29–36]. Several explanations have been advanced for these unusual findings, including neurological causes, subtle clotting, perfusion abnormalities, organizing pneumonia, but no reliable evidence has been provided so far [36–40]. Thus this "hot" topic remains matter of ongoing debate because of its high clinical relevance [29, 30, 39–44]. We hypothesize that histological information regarding the "early" phases of the disease, when a mild to moderate pneumonia is diagnosed in a swab-positive patient, could be crucial to clarify the above described issues, and also in suggesting the best therapy for minimizing disease progression [45].

Perioperative samples

Only a few reports of early/moderate COVID-19 pneumonia are currently available, collected during surgical procedures carried out in patients in which the infection by SARS-CoV-2 was eventually documented (Table 1) [46–50]. In all these studies a pattern of lung injury not corresponding to the typical DAD pattern was reported, with AECII hyperplasia, but without hyaline membranes. Other features included scanty fibrosis, interstitial inflammation, intra-alveolar edema and/or proteinaceous exudates in variable quantity. Abnormal perivascular accumulation of CD4+ helper T-lymphocytes and CD163+ macrophages was described in a single case [49]. Although sparse, these reports can provide relevant information regarding the "missing link" in disease progression, and have raised interest and discussion for their contribute in understanding the early mechanisms of lung injury following SARS-CoV-2 infection [6, 26, 51, 52].

Live diagnostic biopsies

A more systematic description of early/moderate COVID-19 interstitial pneumonia was recently reported by our group, analyzing a series of 12 cases (Table 1) [53]. The patients in this series, recognized by nasal swab and/or bronchoalveolar lavage PCR test as harboring SARS-CoV-2 infection, underwent lung cryobiopsy in the course of the disease (<20 days from symptoms' onset). All patients had a CT

Table 1 Available histological studies of early-phase COVID-19 pneumonia (perioperative samples and biopsies).

Numbers of cases	Disease phase	Sample type	TC imaging	Typical DAD pattern	Organizing changes	Edema exudates	Hyaline membranes	Patchy AECII hyperplasia	Vascular changes	Lymphocyte infiltration	Alveolar macrophages	Concurrent diagnosis	Reference	Country
1	Early	Perioperatory	GGO	0	N.a	N.a.	0	0	N.d.	N.a.	1	Carcinoma	Cai et al. [46]	China
2	Early	Lobectomy	GGO	0	1 AFOP	2	0	2	N.d.	N.a.	2	Carcinoma	Tian et al. [47]	China
1	Early	Lobectomy	GGO	0	0	1	0	1	N.d.	CD8+	1	Carcinoma	Pernazza et al. [48]	Italy
1	Early	Lobectomy	GGO	0	0	1	0	1	Dilatation	CD4+ perivascular	1	Benign nodule	Zeng et al. [49]	China
1	Early	Lobectomy	GGO	0	1	1	0	1	N.d.	N.a.	1	Carcinoma	Cinar et al. [50]	Turkey
12	Early	Live TBB/Cryo	GGO	0	0	Focal	0	12	12 Dilatation	8	12	COVID-19	Doglioni et al. [53]	Italy

GGO ground-glass opacity.

scan documenting typical lung opacities and a variable degree of hypoxemia, but with no needs of intubation and mechanical ventilation. As observed in the perioperative case reports, signs of acute lung injury were present, but without the typical features of the DAD pattern. Edema and cellular debris occurred only occasionally in alveolar spaces, and hyaline membranes were absent in all 12 cases. Interstitial fibrosis was either focal or minimal. *AECII* hyperplasia was heterogeneous and characterized by an unusual "patchy" distribution, with epithelial clusters ranging from isolated small aggregates to wide proliferation of micronodular and/or pseudo-papillary sprouts of AECII, interposed to variable proportions of normally looking type-I pneumocytes (Fig. 1a, b).

The target of SARS-CoV-2 infection

FISH analysis in this series revealed SARS-CoV-2 RNA in a proportion (<10%) of AECII, without any positivity in alveolar macrophages and blood vessels. This finding is consistent with the notion that epithelial cells are the principal target of SARS-CoV-2 infection in both the upper respiratory system and the distal lung [54–56]. AECII steadily express the two key host proteins utilized by the virus to gain entry and replicate within cells: angiotensin-converting enzyme 2 (ACE2) and the cell surface transmembrane protease serine 2 (TMPRSS2) [57]. SARS-CoV-2 infected cells can be detected in tissues by several methods characterized by variable levels of sensitivity and specificity, including molecular in situ analysis, immunohistochemistry, and electron microscopy [58–60]. SARS-CoV-2 infected alveolar-epithelial cells have been consistently detected in most reports using these methods, whereas the infection evidence for other cell types, including endothelial cells and macrophages, is scarce and contradictory [17, 60–62], thus suggesting that infection of cells other than epithelial ones occurs rarely in early-phase COVID-19 pneumonia, and does not represent a major pathogenic mechanism.

AECII in early-phase pneumonia exhibit a high proliferation rate (>50% positive cells) at Ki67-immunostaining, as also observed in DAD [63]. The lack of necrosis or apoptosis (as defined by cleaved caspase-3 analysis) suggests that SARS-CoV-2 infection does not induce widespread epithelial cell death (either mediated by the virus or by cytotoxic cells), at least in early-phase cases, thus explaining the lack of hyaline membranes. Although the evidence that AECII are a target of SARS-CoV-2 infection is generally accepted, little attention has been provided to the fate of these cells after infection, as well as the potential effects on the renewal mechanisms of gas-exchange units. In most acute pulmonary diseases the main targets of damage are either the easily injured type-I pneumocytes,

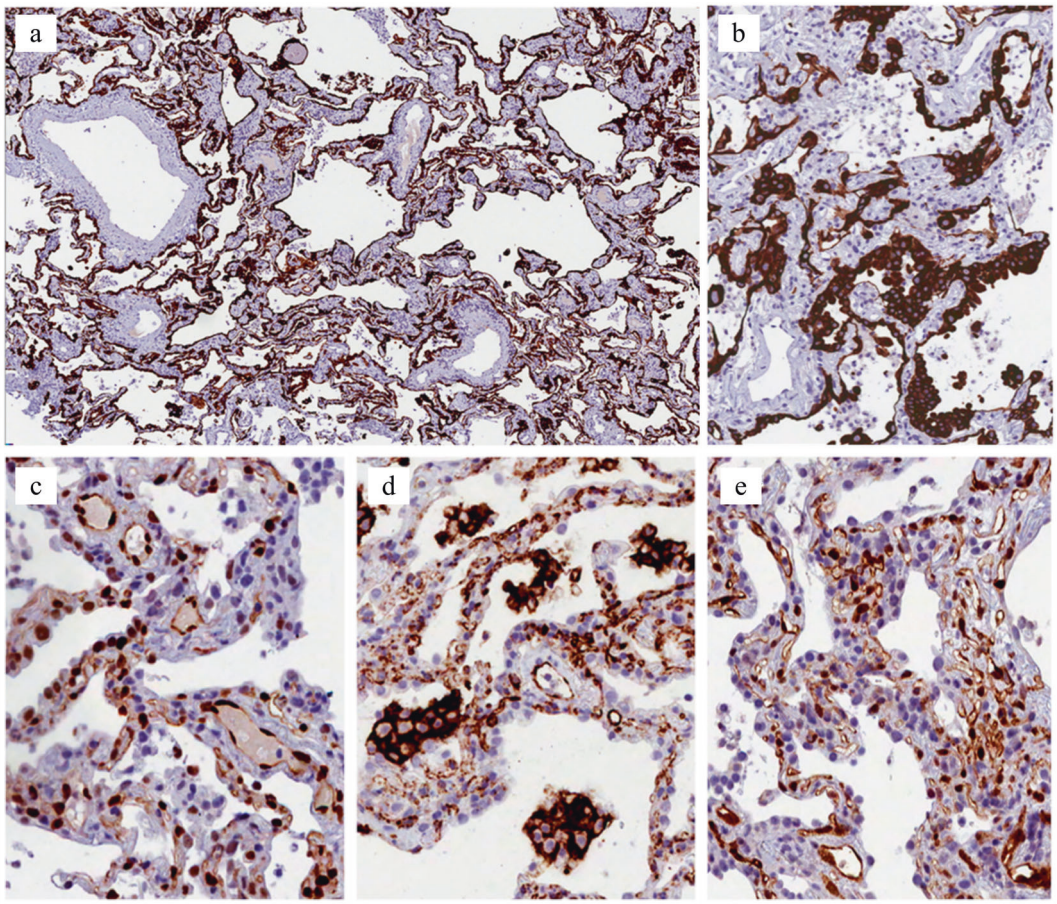

Fig. 1 Early-phase COVID-19 pneumonia. Cytokeratin-7 (CK7) immunostaining provides a better evaluation of the peculiar pattern showing patchy AECII hyperplasia and increased number of enlarged vascular structures with perivascular lymphoid infiltrate (**a**, **b**). No hyaline membranes are evidenced. Diffuse nuclear expression of Tyr705 pSTAT3 immunostaining in activated AECII and blood vessels (**c**). Diffuse PD-L1 expression in blood vessels and intraluminal macrophage aggregates (**d**). Diffuse IDO expression in endothelial cells in both interstitial capillaries and post-capillary venules (**e**).

endothelial cells, or both [64]. The eventually occurring AECII hyperplasia is regarded as a reparative mechanism aimed at replacing the sloughed type-I pneumocytes, which allows a correct balance between epithelial and mesenchymal growth and regulated by a complex array of developmental-related signaling pathways within specialized niches [65]. The lack of hyaline membranes in early-phase pneumonia may suggest that damage of type-I cells does not represent a major event, thus explaining the relative preservation of alveolar structure and gas-exchange functions. In addition, accumulation of collagen and myofibroblasts in interstitial spaces is minimal in early-phase COVID-19, and the sensitive marker of ongoing fibrosis tubulin-beta-3 is either absent or minimal, at variance with typical DAD where strong and diffuse tubulin-beta-3 is expressed by interstitial myofibroblasts [66]. These findings provide relevant histological support to the clinical evidence that COVID-19 acute pneumonia is different from ARDS, as previously discussed.

AECII express factors involved in activation of the "cytokine storm"

Hyperplastic AECII in COVID-19 early-phase pneumonia diffusely express the Tyr705 phosphorylated form of STAT3 (pSTAT3) [53], a master gene product involved in the JAK2/STAT3 pathway and cytokine abnormal production in COVID-19 [67] (Fig. 1c). Similar findings were described in a single postmortem early-phase case by Brown et al. [68]. Interestingly, IL-6 RNA expression was also evidenced by an in situ analysis of cells corresponding for morphology and distribution to SARS-CoV-2 infected AECII [53]. AECII are physiologically committed to produce IL-6 when stimulated by cytokines, and the STAT3 pathway is involved in pneumocyte differentiation [69]. Taken together these findings suggest that after viral infection of pneumocytes a local overproduction of cytokines is triggered within the pulmonary microenvironment, without necessarily increasing their systemic level as

observed in severe COVID-19 [70]. The occurrence of an infection-related shift from STAT1 to STAT3-pathway activation has been recently proposed as central in COVID-19, triggering a cascade of pathogenic events that can explain many features of the disease [71]. In addition, IL-6 is able to activate the STAT3 and NF-kB pathways also in nonimmune cells, including epithelial cells and endothelial cells, triggering a positive feedback loop of NF-κB activation [67, 72, 73]. Amplification of this pro-inflammatory process is likely provided in disease progression by involvement of other cell types (e.g., macrophages), which increase the production and diversification of cytokines leading to an overt DAD. This finding may be relevant to understand the time frame for the proper use of molecules modulating the JAK2/STAT3 pathway [74, 75].

Vascular involvement in COVID-19 pneumonia

There is widespread consensus on considering vascular damage as a central player in the cascade of coagulation abnormalities eventually leading to thromboembolic complications in severely ill COVID-19 patients [76–79]. Vascular dysfunction is associated with vasoconstriction, inflammation, permeability and coagulation abnormalities, and is common in several comorbidities with ominous prognosis in COVID-19, including obesity, older age, diabetes mellitus, hypertension, chronic lung disease, coronary artery disease and heart failure [4, 80, 81]. The infection could decrease the availability of ACE2, thus impairing its counteracting functions on the renin-angiotensin system with eventual alteration of blood pressure and electrolyte homeostasis [82, 83].

Different mechanisms can be hypothesized for the derangement of vascular homeostasis, including different types of SARS-CoV-2 interactions with endothelial receptors. Some evidence for a direct endothelial infection by SARS-CoV-2 has been provided in pulmonary and extrapulmonary sites using electron microscopic images [18, 61], a methodological approach that has been questioned [84–86]. In addition, endothelial infected cells were not reported in postmortem and antemortem studies, where sensitive immunohistochemical and molecular in situ methods were applied [60]. Furthermore, endothelial cells express high amounts of the virus–receptor ACE2, but not TMPRSS2 necessary for spike protein priming [87]. Additional factors involved in endothelial damage and activation include angiotensin-II [88], a factor whose level in the plasma sample from COVID-19 infected patients is elevated and linearly correlated with viral load and lung injury [89]. IL-6 also promotes a sustained loss of endothelial barrier function via JAK-STAT3 [90], and this activity may be particularly effective within the restricted alveolar microenvironment. Indeed, experimental endotheliopathy can be triggered by plasma from severely ill patients [91]. An interesting alternative is provided by the interaction of viral spike proteins with endothelial receptors. In a recent experimental study Suzuki et al. could demonstrate that SARS-CoV-2 spike protein alone, without the rest of the viral components, is able to elicit cell signaling in human host cells [92]. Pathogenic interaction of spike protein with the transmembrane pattern recognition receptor TLR4 (Toll like receptor-4) has been hypothesized in COVID-19 [93]. Along this line, activation of the endothelial TLR4/NF-kB pathway has a critical role in glucose-induced inflammation and damage in diabetes, a condition predisposing to severe complication in COVID-19 [1, 94]. Whatever the triggering cause of endothelial damage, the eventual vascular dysfunction is considered a relevant pathogenic component in the disease severity [95]. The occurrence of vascular damage in COVID-19 is confirmed by the increase of various biomarkers of endothelial damage including circulating endothelial cells and endothelial extracellular vesicles [96, 97].

Endothelial cells express factors involved in the cytokine storm, immune checkpoint and vasodilation

Histological evaluation of early-phase COVID-19 on cryobiopsies [53] revealed relevant abnormalities in pulmonary vessels, which increased in number, showing congestion, dilation and tortuosity (Fig. 1a). According to this report, the thickened edematous walls of post-capillary venules are often infiltrated by CD4+ T-lymphocytes, without overt vasculitis or endothelialitis (Fig. 1a) [53]. A striking finding was the strong and diffuse expression of pSTAT3 in endothelial cells, together with diffuse expression of immune checkpoint molecule Programmed Death-Ligand 1 (PD-L1) and indoleamine 2,3-dioxygenase 1 (IDO) (Fig. 1c–e). These unexpected findings raise a number of considerations that might contribute to a better understanding of COVID-19 pneumonia.

The observed hyper-expression of pSTAT3-Tyr705) not only in AECII but also in endothelial cells in COVID-19 early-phase reinforces the above reported hypothesis of a local, peculiar triggering of this potent pro-inflammatory pathway in the pulmonary microenvironment. Interestingly, the specific phosphor-Tyr705 of STAT3 has been experimentally associated to inflammation and coagulopathy in experimental sepsis [98], as also commonly observed in COVID-19 [18, 37, 78, 99].

Programmed death-ligand 1 (PD-L1)

The robust expression in endothelial cells of PD-L1 and IDO in early-phase/moderate COVID-19 pneumonia

(minimal or even absent in normal lung), may explain in part the severe defects in innate and adaptive immunity observed in COVID-19, as also observed in H9N2 avian influenza virus infection [100]. Experimental evidence has been provided that PD-L1 expression is induced in endothelial cells by interferons and IL-12 [101]. In cancer, the endothelial cells can participate in immune inhibition by immune cell checkpoint signaling, since PD-L1 is highly expressed on endothelial cells and concurs in limiting the infiltration by CD8+ T cells [102, 103]; similar mechanisms are likely occurring in COVID-19. Interestingly, the inhibitory signals exerted by pulmonary endothelial PD-L1 are involved in sepsis and acute lung damage, and are exploited as potential target for therapy of these diseases [104–106].

Further inhibitory signals can be delivered within the alveolar microenvironment by the many monocytes/macrophages expressing PD-L1 which can be demonstrated in early-phase/moderate pneumonia forming endoluminal clusters (Fig. 1d). The nature and role of these macrophages within the alveoli is not clear, since they express an unusual "hybrid" phenotype including dendritic-cell markers such as CD123 and CD205. Lack of expression of CD117 and CD303 excluded other cell types characterized by constitutive expression of CD123/IL3AR (i.e., mast cells and plasmacytoid dendritic cells). Interestingly, a similar phenotype is induced in monocytes by pulmonary epithelial cells that promote TH17 differentiation [107]. The putative inhibitory role of these intra-alveolar macrophages on adaptive immune response following infection was already suggested and related to their expression of immunoregulatory molecules [108, 109]. The role of monocytes and macrophages in the pathogenesis of COVID-19 has been extensively analyzed [110, 111], but the specific significance of PD-L1^{+}, CD123^{+} alveolar macrophages during early-phase pneumonia is far from clear and warrants further investigation.

Indoleamine 2,3-dioxygenase 1 (IDO)

IDO is an intracellular monomeric, heme-containing enzyme, coded by the gene IDO-1, which controls the L-tryptophan breakdown to *N*-formylkynurenine, thus regulating the availability and metabolic activity of this essential amino acid [112]. Tryptophan catabolites have an antimicrobial role, but can also highjack the host immune response in chronic viral infection since they can suppress T-cell responses and promote tolerance [113, 114]. In normal tissues IDO is expressed by dendritic cells in lymphoid organs, and its immune-inhibitory activity is considered of paramount importance for their function [115, 116]. In COVID-19 T-cell lymphopenia and CD8-cell exhaustion are very common findings, and IDO-related tryptophan depletion might be involved in establishing these immune dysfunctions [117, 118].

The endothelial expression of IDO is heterogeneous in different tissues: in human placenta strong IDO activity and expression have been demonstrated and likely exerts a fundamental role in feto-maternal tolerance and vascular tone regulation; IDO insufficiency is involved in intrauterine growth restriction and pre-eclampsia [119]. In the lung, experimental IDO deficiency impairs vascular development, and is able to increase IL-6 during tumor formation [120]. The IDO-mediated mechanisms implicated in vascular tone regulation include the production of kynurenin, a tryptophan catabolite with vascular relaxing activity [121, 122], and also its nitrite reductase activity, likely involved in observed local production of nitric oxide (NO) under anaerobic conditions, as recently demonstrated [123]. The abnormally increased activity of IDO demonstrated by Doglioni et al. [53] is likely responsible for the pulmonary vasodilation and vasoplegia characterizing early-phase/moderate COVID-19 pneumonia, as previously described. This finding is hard to be reconciled with the occurrence of vascular dysfunction, a condition considered a relevant pathogenic mechanism in severe COVID-19 cases. Vascular dysfunction is characterized by reduced vascular relaxation, vasoconstriction, reduced NO synthesis, and increase in angiotensin-II related inflammation and hypertension [122, 124], at variance with the observed increase of vascular enlargement and vasoplegia in early-phase COVID-19 pneumonia, as discussed above [125, 126]. Nevertheless, this complex scenario may be explained by the proposed biphasic presentation of COVID-19. The early type-L pattern (also defined as CARDS—*coronavirus disease-associated acute respiratory distress syndrome*) is characterized by preserved compliance, hypoxemia and out of proportion hypocapnia [29, 127]. In some patients orthodeoxya and platypnea similarly to that observed in well known causes of right to left shunt (patent foramen ovale, hepatopulmonary syndrome) is even clearly documented [128, 129]. These physiopathologic aspects are related to the increased vascular bed (mainly for vascular relaxation) around not-collapsed alveoli [126].

The severe type-H pattern observed in either progressed cases or in a few early cases, on the other hand, is likely characterized by *bona fide* vascular dysfunction, extended alveolar damage leading to alveolar collapse. Vascular relaxation and mechanisms underlying its development, in particular IDO activity, warrant further investigation, since may be a key factor in determining either the positive or

negative evolution of the disease. Indeed, IDO can determine perfusion defects and hypoxia, and activation of the IDO-kynurenine pathway characterizes experimental sepsis-related hypotension, a dramatic state that can be reversed by IDO inhibition [121]. On the other hand, the IDO-kyruneine pathway exerts a protective role against development of pulmonary hypertension and lung damage in experimental studies [130, 131], and this complexity should be carefully taken into account when planning potential therapeutic strategies.

Experimental evidence has been provided for an IDO-dependent suppression of type-I IFNs production in viral infection, and this activity may contribute to the overall pathogenic type-1 IFN deficiency observed in COVID-19 [132]. Evidence for impaired availability of type-1 IFN in COVID-19 is robust and may be supported through different mechanisms [133–136]. Clinical complications observed in COVID-19 may be explained, at least in part, by IDO hyper-expression. For instance, increased IDO-mediated degradation of L-tryptophan has been demonstrated in morbid obesity, a predisposing condition to severe complications in COVID-19 [137, 138].

In most normal tissues endothelial IDO expression is negligible, but it increases after non canonical NF-kB-dependent IFN-γ stimulation and in cancer, where it is involved in tumor resistance to immune response and immunotherapy [112, 139–141]. IL-6 and the STAT3 pathway are also regulators of IDO expression [71, 141], and vascular co-expression of pSTAT3 and IDO in the lung further supports their causative role in COVID-19 pathogenesis. The similarities observed in IDO-related mechanisms in COVID-19 pneumonia and cancer are intriguing, and the evaluation of clinical effects of checkpoint inhibitor therapies in SARS-CoV-2 infected cancer patients may provide future useful information regarding their possible beneficial or detrimental effects on COVID-19 pneumonia [142, 143].

Concluding remarks

All these data taken together confirm the central role of infected AECII in COVID-19. The progressive proliferation of pSTAT3 expressing AECII is nevertheless accompanied by diffuse morphologic and phenotypic abnormalities of the parenchymal blood vessels (dilatation, hyper-expression of pSTAT3, and its targets PD-L1, and IDO), without evidence of endothelial infection. These findings strongly suggest that in COVID-19 a pathogenic cross-talk between epithelial infected cells and endothelial noninfected cells takes place, in agreement with experimental co-culture data [144]. The demonstration of early activation of the STAT3 pathway and its target genes within the alveolar microenvironment may explain a variety of pathogenic features of COVID-19, as previously hypothesized) [71]: 1. the overproduction of plasminogen activator inhibitor-1 (PAI-1) may be involved in the observed derangement of coagulation mechanisms, 2. the robust immune-inhibitory signals exerted by PDL-1 and IDO may contribute to the impairment of cellular immune response, and 3. the physiopathological abnormalities with silent hypoxia related to vasoplegia as previously discussed. Another point to consider is the accumulation in the alveolar spaces of macrophages with an "inflammatory" phenotype; their role is not yet clear possibly representing an attempt to limit the alveolar damage or, viceversa, the trigger that multiply the damage.

The histological features characterizing early-phase/moderate pneumonia finally provide the anatomic ground to interpret the COVID-19 associated CT features, including ground-glass opacities and the "vascular enlargement pattern", a still debated finding with relevant diagnostic potential [126, 145]. The nature of these gravity dependent imaging changes are matter of speculation, but according to available findings they can be reliably ascribed to vascular enlargement, blood stasis and macrophage accumulation, all features that may help in defining diagnostic and prognostic information. Interestingly, imaging abnormalities are also frequent in asymptomatic SARS-CoV-2 infected cases [146, 147], thus suggesting that vasoplegia can occur very early in the disease.

In conclusion, although AECII represent the prevalent target of viral infection and the first trigger of localized IL-6 production and STAT3-pathway activation within the alveolar microenvironment, endothelial cells appear crucial in providing the pulmonary pathophysiological ground to the disease, as shown in the pathogenic scheme here proposed (Fig. 2). This is why, in COVID-19, these two cell components behave as "*victims* and *partners in crime*". This hypothetical pathogenic scheme needs to be validated on larger cohorts, investigating and comparing the morphological and molecular features of the different cell components as observed in early-phase pneumonia with postmortem samples, with the aim to unveil the mechanisms determining the heterogeneity and evolution risks and thus paving the way to personalized therapies in different disease phenotypes [148]. The possible spreading of infection to macrophages within the alveolar microenvironment is a likely candidate for explaining the systemic involvement of cytokine storm and dramatic evolution of disease severity in predisposed patients [149, 150].

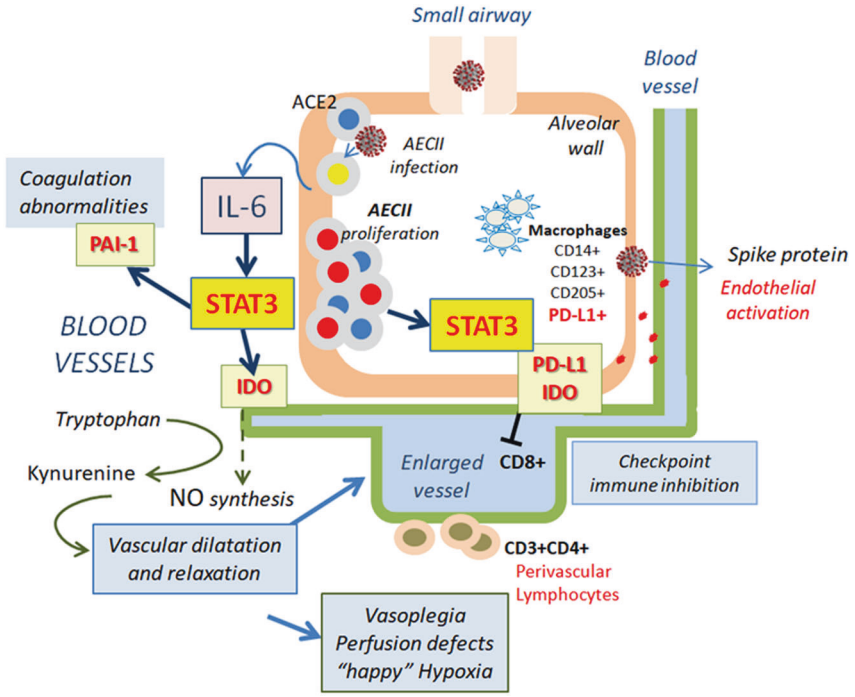

Fig. 2 Hypothetical mechanisms involved in early-phase COVID-19 pneumonia, as discussed in this review. The principal steps include 1. Viral infection of AECII, 2. activation of the STAT3 pathway in AECII, 3. Abnormal STAT3-mediated cross-talk between infected AECII and noninfected endothelial cells, 4. increased expression of PD-L1 (endothelial cells and macrophages) inducing checkpoint immune inhibition of adaptive immune responses, 5. Endothelial hyper-expression of IDO inducing dilation and relaxation of pulmonary vessels, and 6. Diffusion–perfusion mismatch, shunting, pathophysiological changes, absence of alveolar collapse, vasoplegia, and "happy hypoxia".

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Compliance with ethical standards

Conflict of interest The author declares no competing interests.

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