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Review Article

Aspects of immunopathology in humans during ssRNA virus infections - what do we know and do we have a foundation for treatment? - A narrative review - Part One.

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Abstract

Background: The pathophysiological mechanisms underlying single-stranded RNA (ssRNA) virus infections have been investigated extensively and at an accelerated pace in recent years, largely driven by the COVID-19 pandemic. Particular attention has been given to their potential roles in respiratory insufficiency, endothelial dysfunction, thrombosis, and inflammatory responses within the central nervous system.
Aim: This narrative review aims to summarize important pathophysiological mechanisms and pathways reported in the literature on respiratory ssRNA virus infections. Key findings are used to explain the outcome of present treatments and to suggest a more immuno-pathological based treatment strategy in these patients.
Method: The review is based on studies extracted from an extensive body of literature, encompassing basic research in biochemistry, immunology, virology, and genetics, as well as clinical and therapeutic studies, all indexed in the PubMed database.
Results: A species specific human immunological pathway reaction occurs against respiratory ssRNA viral genome antigen. It encompasses by an inferior interferon response limiting the force of the viral defense. A viral independent immunoinflammatory response, with monocytic cell infiltration in the lung ensues and may become severe, characterized by fortifying endothelial activation, a prothrombogenic pathway, and respiratory insufficiency. The pro-inflammatory response has potential ability of becoming chronic, with propagation to the central nervous system, but all responses seem potential treatable from an immune-pathological perspective.
Conclusion: The review may serve as a template for treatment and for future studies. It can be concluded that it is essential to have broad immunopathological knowledge when treating and developing strategies for ssRNA virus infections.

Keywords : *RNA virus, innate immunity, COVID-19, RSV, Influenza.*

Introduction

The COVID-19 pandemic caused a significant increase in research activity to understand the pathophysiological mechanisms that are involved in ssRNA virus infections. This research has progressively been published in a vast numbers of articles and journals, established an extensive body of literature, encompassing basic research in biochemistry, immunology, virology, and genetics, as well as in clinical specialties. The amount of published research has made it difficult to obtain and digest all relevant information. In particular, it has been difficult to achieve insights into all relevant key host defense mechanisms and recognize comprehensive patterns of response that may be of clinical importance.

Single-stranded RNA (ssRNA) viruses, such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), respiratory syncytial virus (RSV), and influenza virus are highly contagious viruses. They are all associated with clinical symptoms from the respiratory tract, ranging from mild symptoms to life threatening respiratory insufficiency. Infections with SARS-CoV-2, influenza and RSV can progress to critical illness with hypoxemia, dyspnea and acute respiratory distress syndrome (ARDS), all of which are associated with a high risk of mortality [1, 2]. SARS-CoV-2 causes the COVID-19 pandemic, which is considered to have the highest transmission rate (4). It spread globally in less than a year despite extensive public health measures, including quarantines in some countries, and was

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associated with a high risk of mortality.

The purpose of this narrative review is to describe and summarize important pathophysiological pathways connected with ssRNA virus infections, with a main focus to describe the sequence of events in an understandable overview. The review, which is divided into two parts, is based on articles within several subjects of interest extracted from hypothesis generated studies and an extensive body of literature, encompassing basic research in biochemistry, immunology, virology, and genetics, as well as clinical and therapeutic studies, trying to define and describe the pro-inflammatory response in humans. Furthermore, the review provides insights into the mechanisms underlying the high incidence of pulmonary thrombosis observed in affected patients, the development of multisystem inflammatory syndrome in children (MIS-C), and the post-infectious cerebral inflammatory conditions, which may occur in affected patients. In part two it discusses present treatments and ends with a suggestion of an immune-pathological oriented treatment strategy.

Single-stranded RNA viruses (ssRNA)

Single stranded RNA viruses contain a single RNA genome and comprise many virus families. This review recognizes a few, which cause respiratory symptoms and are common occurring in the society.

ssRNA viruses can either contain a positive-sense RNA genome (+ ssRNA) or a negative-sense RNA genome (− ssRNA).

In + ssRNA viruses, the RNA genome resemble messenger RNA (mRNA) and can directly be translated into viral proteins by the ribosomes in the infected cells. Coronaviruses (e.g., SARS-CoV-2) and rhinoviruses are typical +ssRNA viruses, responsible for COVID-19 and the common cold, respectively. In − ssRNA viruses, the RNA genome serves as a template for mRNA synthesis. This process requires the presence of the enzyme, RNA-dependent RNA polymerase, which is packaged within the virus capsid and when it is released into the infected cell transcribe the negative RNA genome to mRNA. Respiratory syncytial virus (RSV) and influenza viruses are representative −ssRNA viruses, both of which cause respiratory distress in humans.

The spread of ssRNA viruses

Since ssRNA viruses, such as SARS-CoV-2, rhinovirus, respiratory syncytial virus (RSV), and influenza virus contain only a single RNA genome they are lighter than e.g. DNA viruses. This can partly explain why they are highly contagious. SARS-CoV-2 is considered to have the highest transmission rate [3]. It spread globally in less than a year despite extensive public health measures, including quarantines in some countries.

The transmission of the ssRNA virus, RSV, was investigated by Kulkarni et al. [4]. They demonstrated that children with RSV bronchiolitis exhaled large quantities of virus particles. The virus occurred in small aerosol particles enough to remain airborne for several hours, even in intensive care rooms with an air exchange rate of 10 times per hour. Viable RSV was detected in these airborne aerosol particles up to five meters away from infected children two hours after exhalation, as confirmed by the virus's ability to infect human epithelial cells. These insights into RSV aerosol transmission in well ventilated rooms help to explain the rapid global spread of SARS-CoV-2. It also endorses that the main transmission of these respiratory viruses are airborne and that long-distance transmission of up to 7-8 m occurs, as has been published in case reports [5, 6].

The entry of ssRNA viruses into the infected host cells

After inhalation, ssRNA viruses utilize various mechanisms to attach to virus-specific receptors on respiratory epithelial host cells, triggering distinct processes that enable entry into the host cell. Although, respiratory epithelial cells are infected, resident alveolar macrophages, which are regarded to be the first line defenders of the alveoli, appear unable to mount a response to SARS-CoV-2 [7]. The virus–receptor complex then triggers viral entry into intracellular vesicles called endosomes. The exact mechanisms vary among ssRNA viruses and depend on the nature of the virus–receptor interaction. Viral genomes are released into host cells either directly at the plasma membrane or through endocytic pathways following receptor engagement [8]. These pathways include clathrin-dependent endocytosis, clathrin-independent endocytosis, caveolin-mediated endocytosis, and macropinocytosis [9]. Clathrin-dependent endocytosis is a process where the cell membrane invaginates and forms a vesicle. This process involves the protein clathrin there of the name. The caveolin-mediated endocytosis is independent of clathrin, but formed by the protein caveolin and rich in cholesterol and sphingolipids. Macropinocytosis is an actin-dependent non-selective uptake and internalizes a large part of the surface membrane including all its receptors into vesicles called macropinosomes and late-endosomes.

SARS-CoV-2 targets the respiratory tract cells by binding to angiotensin-converting enzyme 2 (ACE2), initiating clathrin-mediated endocytic entry into primarily lung epithelial host cells [10-15]. This interaction also inactivates ACE2's normal physiological function, namely, the degradation of angiotensin II (ANG II), a key component of the renin-angiotensin-aldosterone system (RAAS) [12].

RSV binds to growth factor receptors and initiates macropinocytosis-mediated entry, with nucleolin acting as a cofactor [16-18]. Since macropinocytosis internalizes

large portions of the cell membrane along with associated receptors, it may account for the observed early depletion of ACE2 and decreased degradation of ANG II during RSV infection [19].

Influenza viruses enter cells through multiple routes, including clathrin-dependent and clathrin-independent endocytosis, and have also been shown to downregulate ACE2 expression. This leads to a decreased degradation of ANG II, demonstrated both in experimental models and in human infections [20-23].

Despite differences in entry mechanisms, all three ssRNA viruses, SARS-CoV-2, RSV, and influenza virus, can cause severe respiratory distress in humans and all three have been shown to reduce ACE2 levels. This reduction in ACE2 may contribute to increased ANG II levels in the lungs. Elevated levels of ANG II are correlated with disease severity in patients with SARS-CoV-2, influenza and RSV infections and delivery of ACE2 has been protective [19, 20, 24, 25].

The endosomal toll-like receptors (TLR) 7 and 8 recognize ssRNA virus

Endosomes, into which viruses are internalized during cell entry, contain a class of pathogen recognition receptors (PRRs) called toll-like receptors (TLRs). These specific endosomal TLRs are named TLR3, TLR7, TLR8, and TLR9 and recognize viral nucleic acids. They play critical roles in the immune response to different types of viruses. TLR3 responds to double-stranded RNA (dsRNA) viruses, TLR7 and TLR8 detect single-stranded RNA (ssRNA) viruses, and TLR9 recognizes double-stranded DNA (dsDNA) viruses [26].

Although both TLR7 and TLR8 respond to ssRNA viruses, they exhibit important species-specific differences, cell type-specific expression patterns, and distinct downstream immune responses [27-29]. Heil et al. demonstrated that ssRNA viruses are detected primarily via TLR7 in murine models and via TLR8 in humans [30]. The absence of a significant TLR7 response in humans has been reported by several [31-33]. Conversely, in mice, ssRNA viruses predominantly engage TLR7, with TLR8 showing little or no activity. This is one reason why the TLR8 pathway has been underexplored in murine studies [34, 35].

In humans, TLR8 is expressed in various cell types, including pulmonary epithelial cells, macrophages/monocytes, neutrophils, myeloid dendritic cells, and regulatory T cells [28, 36, 37]. TLR7 expression in humans occurs predominately in plasmacytoid dendritic cells and to a lesser extent in B-cells and monocytes [27, 34].

Differences in TLR 7 and TLR 8 response pathways

Both TLR7 and TLR8 signal through the myeloid differentiation primary response 88 (MyD88) adaptor protein. Despite sharing this common adaptor, the two receptors activate different signaling cascades that result in distinct profiles of inflammatory cytokines, chemokines, and transcription factors [28, 34, 38].

TLR7 is a relatively weak inducer of cytokines and chemokines compared to TLR8. It is a strong and critical activator of type I interferons, particularly interferon-α (INF-α), and to a lesser extent, interferon-β (INF-β), through activation of interferon regulatory factor 7 (IRF7) [39]. Type I interferons are essential in murine models for maintaining robust antiviral defenses and for supporting the survival of memory B cells, thereby contributing to long-lasting immunity in murine [40].

TLR8 is a significantly stronger inducer of inflammatory cytokines and chemokines than TLR7 in experimental models. Monocytes stimulated via TLR8 produce approximately 100 times more cytokines than when stimulated via TLR7 agonists. TLR8 is a weaker inducer of type I interferons and primarily induces INF-β via activation of interferon regulatory factor 3 (IRF3) [27, 28, 41]. Notably, IRF3 activation is inhibited by SARS-CoV-2 membrane proteins, which block the production of INF-β [42]. In humans, the TLR7 response to ssRNA viruses is weak and limited to a subset of plasmacytoid dendritic cells that produce INF-α [34, 43, 44].

The underlying mechanism of the differences in the amount of cytokines and chemokines as in interferon signaling pathways may be caused by the MyD88-IRF interaction [38]. Additionally, in humans, ssRNA viruses induce the production of leukotriene B4, prostaglandin E2, and platelet-activating factor through the TLR8 pathway, not the TLR7 pathway [45].

COVID-19 is characterized by the absence of INF-β and reduced levels of INF-α, consistent with a dominant TLR8-mediated response [43, 46]. This supports the observation of a suppressed type I interferon response during ssRNA virus infections in humans.

Human epithelial cells also appear to mount a delayed interferon response to SARS-CoV-2 through the double-stranded RNA sensor MDA5 (melanoma differentiation-associated gene 5). The absence of IRF7 in these cells suggests also that TLR7 is not activated in human epithelial tissue [47]. This deficiency in type I interferons and the subsequently impaired endogenous antiviral response may contribute to the persistence of SARS-CoV-2 within host cells and the virus ability to evade intracellular degradation [48]. Furthermore, the inability of non-immune cells to produce latent RNases during ssRNA virus infections may explain why these viruses escape degradation in epithelial cells [49].

An adequate INF-α response is essential for antiviral defense and for protecting memory B cells from apoptosis. Therefore, a lack of type I interferons during ssRNA virus infections may partly explain the short-lived immunity observed in humans [40, 50]. This is consistent with findings that antibody responses—such as IgM and IgA—last less than six months, while IgG levels decline after one year following infections

with ssRNA viruses like SARS-CoV-2, RSV, rhinovirus, and influenza virus [41, 51]. Another contributing factor to short-lived immunity may be the delayed presentation of viral genetic material by epithelial cells, which can lead to apoptotic deletion of circulating memory T cells [52].

Important pathological findings in fatal COVID19

Dorward et al. analyzed tissue samples from patients who died of SARS-CoV-2 infection, mapping the presence of the virus and its transcriptional activity across various organs, and correlating these findings with signs of inflammation, thrombosis, and tissue damages [53]. They identified SARS-CoV-2 viral RNA and subgenomic mRNA in all sampled organs and tissues (n = 37), indicating widespread systemic dissemination of the virus during infection in the human body.

Although viral detection and transcription were evident in extrapulmonary organs and tissues, extensive inflammatory responses were observed only in the lungs and reticuloendothelial system. Despite frequent detection of the virus in the kidneys, gastrointestinal tract, liver, and heart, no signs of inflammation or vasculitis were identified in these organs.

In the lungs, the authors observed pronounced inflammation, alveolar damage, and pulmonary vasculitis with thrombi. Importantly, these changes occurred independently of viral RNA or transcriptional activity. A detailed evaluation of the pulmonary vessels revealed virus-independent vascular inflammation, characterized predominantly by calprotectin positive monocytes adherent to the pulmonary endothelium. There was no correlation between evidence of endotheliitis and the presence of virus in endothelial cells. In fact, some regions of pulmonary endothelium were infected with the virus without showing signs of endotheliitis, reinforcing the conclusion that the virus was not the cause of the observed pulmonary vasculitis. The findings of predominantly pulmonary damages have also been reported by others during postmortem examinations of patients with COVID-19 [54].

These findings challenges the hypothesis proposed by Vargas et al., which suggested that endotheliitis was a direct consequence of an endothelial viral infection. However, that study did not clearly state whether pathological analysis and viral detection were performed on the same tissue sample [55]. Moreover, Dorward et al. found no association between viral presence in endothelial cells and the formation of thrombi in pulmonary vessels.

A characteristic histological feature of the lungs in COVID-19 patients is the infiltration of calprotectin producing CD14+ and CD16+ monocyte-derived macrophages [56]. These highly pro-inflammatory macrophages have also been identified in bronchoalveolar lavage fluid from patients with severe COVID-19 [57]. The presence of infiltrating monocytes/macrophages in lung tissue, along with the expression of a pro-inflammatory gene signature, underscores the central role of these cells in immune system activation within affected lungs [58].

In summary, a virus-independent immunopathologic process governed by a non-specific T-cell to endothelial interaction occurs in the lung of patients with critical COVID-19. The non-cytotoxic behavior of SARS-CoV-2 supports the view that it is the recruited monocytes, which convert to macrophages in the lung tissue that is the basis for the life-threatening inflammation and the respiratory insufficiency. It may explain why no specific antiviral therapy has consistently reduced all-cause mortality in patients with severe and established COVID-19 [59, 60].

T-cells and monocytes/macrophages activation

The CD80/86-CD28 pathway activates T-cells

In addition to the MyD88 pathway, the activation of TLR8 strongly induces the expression of CD80/86 on cells infected with ssRNA viruses [61]. The ligand for CD80/86 on the surface of infected cells is the CD28 receptor, which is constitutively expressed on T cells and provides essential signals for T cell activation, proliferation, differentiation, and survival [62-67]. Pro-inflammatory T cells differentiate into helper T cells ($CD4^+$) and cytotoxic T cells ($CD8^+$), while anti-inflammatory T cells differentiate into regulatory T cells (Tregs). The activation of the T-cells, with binding of CD28 to CD80/86 also leads to the upregulation of the CD40 ligand (CD40L), also known as CD154 on the surface of T cells [68]. The importance of the CD80/86 pathway in COVID-19 is confirmed by the finding that blocking it highly antagonizes the pro-inflammatory response elicited by the SARS-CoV-2 virus [62].

The CD40L on T-cells binds to CD40

The ligands for CD40L on activated T cells are CD40 and integrin receptors, which are constitutively expressed on B cells, monocytes, macrophages, dendritic cells, endothelial cells, epithelial cells, and thrombocytes [69-71]. The upregulation of CD40L on T-cells and its binding to cells with CD40 receptors play a broad and critical immunological role in vivo, linking the immune system to the coagulation system, cardiovascular regulation, and microglia in the central nervous system. This interaction requires a tight regulation to maintain a controlled homeostasis [72, 73].

The impact of the CD40L /CD40 interaction with endothelial cells

The CD40L on activated T cells binds to CD40 on endothelial cells, regardless of whether the endothelial cells are

infected or not, since CD40 is constitutively expressed on endothelial cells. Since the T-cells are activated within the lung it is predominantly within the pulmonary circulation they bind to and activate endothelial cells. This promotes a pro-thrombotic state within the pulmonary microcirculation [74, 75]. The CD40L/CD40 interaction represents a critical link between inflammation and coagulation in both acute and chronic inflammatory conditions [75, 76]. Endothelial activation by the CD40L/CD40 interaction leads to the production of reactive oxygen species, metalloproteinases, and tissue factor, as well as upregulation of adhesion molecules such as ICAM-1, VCAM-1, and E-selectin [76-79]. It also induces the release of von Willebrand factor (vWF) multimers from human endothelial cells, thereby contributing to the pro-thrombotic state [80, 81]. Moreover, CD40L on T cells activates platelets, which in turn upregulate CD40L expression on their own surfaces. This feedback loop enhances binding of thrombocytes to the endothelial cells, further endothelial activation, which contributes to adhesion of pro-inflammatory cells and the pro-thrombotic state [82]. The concurrent release of metalloproteinases, particularly ADAM-10, facilitates the cleavage of CD40L from cell surfaces, thereby increasing levels of soluble CD40L (sCD40L) [83-85]. Additionally, the detection of P-selectin, fibrinogen, and soluble CD40L (sCD40L) in patients with SARS-CoV-2 infection further supports a role for CD40L/CD40 signaling in mediating interactions between the immune system, platelets, and the endothelium [86].

Amplification of the pro-thrombotic response by ANG II

The CD40L/CD40-mediated pro-thrombotic response is further amplified by ANG II, a product of RAAS, which is known to increase in several ssRNA virus infections [74, 75, 87, 88]. ANG II induces the expression of tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1) in endothelial cells, thereby promoting thromboembolic events [89-92]. Angiotensin receptor blockers (ARBs), which antagonize the effects of ANG II, exhibit antithrombotic properties [93]. Since SARS-CoV-2 directly inactivates ACE2, which impairs degradation of ANG II, it may potentially explain why there is a higher incidence of thrombotic events in SARS-CoV-2 infection compared to other ssRNA virus infections, such as influenza and RSV [94]. Elevated ANG II levels in COVID-19 patients are associated with increased disease severity [24, 25].

Findings supporting an important role of the CD40L/CD40 interaction

Infiltration of T-cells, mononuclear cells and thrombus formation within pulmonary vessels characterized by histopathological evidence of an inflammatory response that is not associated with viral antigen or viral replication supports a mechanism such as the viral independent CD40L/CD40 signaling pathways [53, 95-98]. Elevated levels of sCD40L are independently associated with increased severity and mortality in COVID-19 [62, 85]. In addition, elevated vWF levels correlate with disease severity and increased mortality in patients with COVID-19 [99].

Clinical implications of anti-thrombotic treatment during CD40L/CD40 interaction

CD40L/CD40-dependent microvascular thrombi are resistant to activated protein C, hirudin, tissue factor inhibition, and heparin, but remain sensitive to antithrombin III treatment, paralleling clinical findings in COVID-19 [90, 100]. The pro-thrombotic effects of CD40L/CD40 signaling are also partially resistant to conventional antiplatelet therapy [101]. This agrees with the finding, that SARS-CoV-2 infection is associated with a high incidence of thrombotic complications, particularly pulmonary vessel thrombosis, even in patients receiving standard thromboprophylaxis, including heparin [53, 100, 102-104]. These observations underscore the importance of monitoring and normalizing antithrombin III levels, and support the use of antithrombin therapy to mitigate thrombotic risk in COVID-19 and other ssRNA virus infections.

Infiltration in the lung with calprotectin releasing cells

The activated T-cells and endothelial cells secrete cytokines and different adhesion molecules, including granulocyte-macrophage colony-stimulating factor (GM-CFS), which stimulates production of granulocytes and monocytes. These cells are recruited to the lung and contribute to the infiltration of calprotectin releasing granulocytes and mononuclear cells [53, 105]. Calprotectin, the active component of the so called MRP8/14 complex, activates β2-integrins, thereby promoting neutrophil rolling and adhesion and amplifying the inflammatory response [106]. Elevated calprotectin levels correlate with disease severity and mortality in SARS-CoV-2 infection, suggesting that the extent of mononuclear cell activation is of clinical relevance in COVID-19 [105, 107-112]. In summary, respiratory ssRNA virus infections are characterized by a mononuclear cell-mediated infiltration, which is independent of the localization of the virus. This mechanism orchestrates a form of immunologic driven endotheliopathy associated with a pro-thrombotic response that is partially resistant to heparin and antiplatelet agents. The pathological response includes T-cells activation followed by endothelial activation, platelet activation, and the production of adhesion molecules and von Willebrand factor [84].

The infiltration, polarization and importance of monocyte-derived macrophages in the lung

During an acute respiratory ssRNA infection, the innate immune system contributes via the CD40L-CD40 adhesion

to the accumulation of a high number of T cells within the lungs. These T cells expand and proliferate into clusters that express the pro-inflammatory cytokines interleukin-17 (IL-17), interferon-γ (INF-γ), and granulocyte-macrophage colony-stimulating factor (GM-CSF) [113, 114]. These cytokines, in turn, stimulate the production of several chemokines, such as INF-γ-inducible protein 10 (IP-10), and other chemo-attractants that recruit monocytes and neutrophils to the site of inflammation [115, 116]. The CD40L/CD40, T-cells to endothelial interaction, promotes thus recruitment and infiltration of monocytes and neutrophils to the lung parenchyma [94]. The pulmonary recruitment and accumulation of monocytes is further enhanced under hypoxic conditions [87, 114, 117]. Most resident alveolar macrophages are depleted and replaced by monocyte-derived macrophages, coinciding with the activation of $CD4^+$ and $CD8^+$ T cells [57, 118]. Resident alveolar macrophages are hyporesponsive to e.g. SARS-CoV-2, exhibiting only weak pro-inflammatory responses to viral infections [7, 119]. This supports the notion that the resident macrophages primary function is the regulation of pulmonary surfactant homeostasis and hemostasis rather than taking direct part in the antiviral defense [120]. These findings align with pathological observations showing a scarcity of tissue-resident alveolar macrophages and an abundance of calprotectin-producing monocyte-derived macrophages in lung parenchyma during acute respiratory viral infections [53, 118].

The recruited monocytes/macrophages are polarized either to pro-inflammatory macrophages (M1) or anti-inflammatory macrophages (M2) governed by at least three well established pro-inflammatory mediators, ANG II, INF-γ, and PPAR-γ [32, 121, 122]

Following an acute viral infection the innate immune response normally transition to an adaptive immune response and a resolution of the local inflammation within one week, when specific antibodies against the antigen occurs. This resolution phase, which involves the clearance of dead neutrophils, cellular debris, and pathogens, depends in part on the polarization of macrophages toward an anti-inflammatory, phagocytic M2 phenotype, due to low levels of ANG II, INF-γ, and high levels of PPAR-γ.

During severe respiratory ssRNA virus infections, caused by respiratory syncytial virus, influenza virus, and coronaviruses the recruited CD14+ and CD16+ monocyte-derived macrophages may polarize toward a pro-inflammatory monocytic/macrophagic phenotype M1, due to abiding high levels of ANG II, INF-γ, and low levels of PPAR-γ [123, 124]. This is in part caused by respiratory ssRNA viruses ability to inhibit ACE2, and thereby increase the local level of ANG II in the lung.

The M1 macrophages become highly activated when their Fc receptors bind to antibody-antigen complex, which especially occurs when the specific IgG production starts after the first week. This may explain the two phases of infection in patients with severe Covid-19. The M1 macrophages are stimulated to produce pro-inflammatory cytokines, chemokines, nitric oxide synthase, calprotectin, and pro-fibrotic mediators, contributing to further neutrophil and immune cell infiltration and damage to the lung, while inhibiting the virus [116, 121, 125, 126]. The production of INF-γ, calprotectin, reactive oxygen species (ROS), metalloproteinases, cytokines, and chemokines by the macrophages is further amplified by ANG II [127].

Persistently elevated levels of ANG II, sustained INF-γ production and reduced PPAR-γ activity may maintain M1 polarization, perpetuating inflammation, lung injury and respiratory distress. Although M2 macrophages are anti-inflammatory, in the context of severe lung damage, they may promote fibrosis, structural disruption, and thereby also respiratory failure [128].

The correlation between calprotectin levels and severity of the disease suggests a significant role by lung-derived pro-inflammatory cells in the pathogenesis [105, 107, 112]. The beneficial effects on the course of the respiratory insufficiency by inhibiting both AT1R (ANG II), JAK-blockade (INF- γ) and/or stimulating PPAR- γ indicate that the polarization of the macrophages is of importance. In summary, ssRNA virus infections cause an innate immune response, which includes an unspecific adhesion of activated T-cells to the endothelial cells in the microcirculation of the lung. These cells initiates normally a well balance pro-inflammatory response to the viral infection, but in some patients the response accelerates, partly orchestrated by ANGII, INF-γ, and lack of PPAR-γ, causing a life-threatening inflammatory response with pro-inflammatory macrophages invading the lung, lung tissue damages and increased risk for thrombosis.

The impact of ANG II on the lung damages

The RAAS regulates systemic vascular resistance, fluid and electrolyte balance, and ultimately maintains blood volume and blood pressure. In addition to these homeostatic roles, RAAS also modulates innate and adaptive immune responses, primarily through its key effector molecule, ANG II [129].

RAAS comprises several components, beginning with angiotensinogen, an acute-phase protein synthesized by the liver [130]. Angiotensinogen is converted into angiotensin I (ANG I) by the enzyme renin, which is secreted as prorenin by the juxtaglomerular cells of the kidney. Renin release is primarily regulated by renal blood flow. During inflammatory states with plasma leak or reduced renal perfusion, both angiotensinogen production and its conversion by renin to ANG I are upregulated.

ANG I is rapidly converted to ANG II by angiotensin-converting

enzyme (ACE), which is abundantly expressed on endothelial cells in the pulmonary capillaries [131, 132]. Although ANG II has a short plasma half-life of less than one minute, binding to its primary receptor, angiotensin II type 1 receptor (AT1R) results in receptor internalization which significantly prolongs its biological activity [133]. ANG II is metabolized either by angiotensin-converting enzyme 2 (ACE2) into angiotensin 1–7 or by aminopeptidases into angiotensin III and IV in red blood cells and peripheral vascular beds [134-137]. In addition to its vasoconstrictive and pro-inflammatory actions, ANG II also stimulates the secretion of aldosterone, further contributing to sodium retention in an attempt to expand a reduced blood volume [132].

Several lines of evidence indicate that RAAS is modulated by various single-stranded RNA (ssRNA) viruses, including SARS-CoV-2, respiratory syncytial virus (RSV), and influenza A. All of these viruses inhibit the function of ACE2 through different mechanisms [19, 138-140].

A decrease in ACE2 activity results in the accumulation of ANG II in the respiratory tract, promoting activation of AT1R. High levels of ANG II, associated with disease severity and strongly correlated with mortality, have also been reported in serum during SARS-CoV-2, respiratory syncytial virus (RSV), and influenza infections [19-21, 24, 25].

Normal levels of ANG II have been observed in patients with mild illness sampled early in the course of the illness at emergency departments [141, 142]. Since ANG II are primarily regulated by circulating levels of ANG I, elevated ANG II levels in serum are more likely to occur during conditions which elevate the levels of ANG I. This may typically occur when the hemodynamic is compromised, such as during systemic inflammation, when an acute phase response produces angiotensinogen and when the circulatory blood volume is decreased due to plasma leak and a subsequent reduction in renal blood flow activates renin. From an evolutionary standpoint, the local production and amplification of ANG II may enhance the immune defense in the lung during life-threatening respiratory infections in situation when the systemic circulation is compromised.

The impact of ANG II on the immune response

The activation of the AT1R by ANG II in the lung contributes to the polarization of the recruited monocyte-derived macrophages toward a pro-inflammatory M1 phenotype. This also leads to the recruitment and accumulation of neutrophils in the lungs [121, 125]. In addition, ANG II stimulates the generation of reactive oxygen species (ROS) [143-146], contributes to pulmonary vascular leak syndrome [147, 148], and promotes the migration of macrophages and dendritic cells [149, 150]. ANG II also exacerbates CD40L/CD40-mediated vascular inflammation and possesses pro-thrombotic properties [75, 88, 90]. It induces the nuclear factor kappa B (NF-κB) pathway, leading to upregulation of cytokines and chemokines [151], and enhances the activity of metalloproteinases such as ADAM17, which shed cytokines and chemokines [152, 153]. Furthermore, ANG II increases the synthesis of endothelial-derived mediators including macrophage colony-stimulating factor (M-CSF), monocyte chemoattractant protein-1 (MCP-1), E-selectin, vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) [154].

ANG II also contributes to thrombocytopenia [145], and enhances vasoconstriction by promoting the release and action of norepinephrine [155], endothelin [156], vasopressin [157], and aldosterone [158]. Moreover, it exerts pro-fibrotic effects in the lungs [159-161] and downregulates anti-inflammatory transcription factors such as PPAR-γ, which further promotes the polarization of monocyte-derived macrophages toward the pro-inflammatory M1 phenotype [151].

The impact of interferon-γ (IFN-γ)

Interferon-γ (IFN-γ) is primarily produced by activated pro-inflammatory T cells ($CD4^+$, $CD8^+$) and natural killer (NK) cells during viral infections and plays a key role in macrophage/monocyte activation and the host defense against infections [162]. IFN-γ exerts its effects through interaction with its receptors, IFN-γ receptor 1 and 2 (IFNGR1 and IFNGR2), which are associated with Janus kinase 1 (JAK1) and Janus kinase 2 (JAK2), respectively. Upon receptor activation, JAK1 and JAK2 phosphorylate tyrosine residues on the transcription factor STAT1. Phosphorylated STAT1 molecules form homodimers, which translocate to the nucleus and bind to gamma-activated sequence (GAS) elements, initiating IFN-γ-regulated gene transcription. In parallel, JAKs also activate phosphatidylinositol 3-kinase (PI3K), which signals through protein kinase C to phosphorylate serine residues on STAT1. Both tyrosine and serine phosphorylation are required for a full IFN-γ response via GAS elements [163].

The JAK–STAT1 signaling pathway mediates multiple inflammatory processes. It enhances the activation of T and B cells, which participate in the respiratory immune response to infection, and also promotes further IFN-γ production in a positive feedback loop [123, 162]. IFN-γ is a potent inducer of major histocompatibility complex (MHC) class II expression on antigen-presenting cells (APCs), thereby improving their ability to present antigens to T cells [164]. It also upregulates CD40 expression on various cell types, including microglia in the central nervous system (CNS), increasing their sensitivity to both membrane-bound and soluble CD40L from activated $CD4^+$ T cells [165].

In addition, IFN-γ regulates the polarization and differentiation

of monocyte-derived macrophages toward the pro-inflammatory M1 phenotype in the lungs [123, 162, 166]. Lung biopsy studies in patients with COVID-19 have demonstrated robust IFN-γ activation, which correlates with disease severity, mortality, and the intensity of the inflammatory response [32, 167-169]. M1 macrophages in the lungs are strongly associated with poor outcomes in COVID-19 [170].

In combination with tumor necrosis factor-α (TNF-α), IFN-γ is a potent inducer of inducible nitric oxide synthase (iNOS), which produces high levels of nitric oxide. Excess nitric oxide is implicated in myocardial depression and hypotension during septic shock [171, 172].

In cancer biology, IFN-γ has a dual role: at high level, it promotes anti-tumor immunity and tumor regression, while at low level it may facilitate immune evasion and tumor progression [162, 163].

Severe multisystem inflammatory syndrome in children (MIS-C) is also characterized by elevated IFN-γ levels and is associated with symptoms such as hypotension and mild-to-moderate cardiac dysfunction, likely mediated by iNOS activation [173]. IFN-γ-driven macrophage polarization may also contribute to macrophage activation syndrome (MAS) observed in MIS-C [174-176]. High levels of IFN-γ have been linked to gastrointestinal manifestations including enterocolitis and right-sided abdominal pain, mimicking acute appendicitis [177-181]. Additionally, IFN-γ contributes to consumptive anemia and induces chemokines such as CXCL9, CXCL10, and CXCL11, which are associated with skin manifestations seen in MIS-C and resemble those in systemic juvenile idiopathic arthritis (Still's disease) [182].

In summary, these findings suggest that elevated IFN-γ levels play a central role in the pathogenesis, especially in patients were respiratory ssRNA infections lead to severe and life threatening disease.

The impact of peroxisome proliferator-activated receptors (PPARs)

Peroxisome proliferator-activated receptors (PPARs), comprising PPAR-α, PPAR-β/δ, and PPAR-γ, are nuclear transcription factors that regulate glucose and lipid metabolism, cell differentiation, and play essential roles in immune modulation [183]. The immunological relevance of PPARs, particularly PPAR-γ has been emphasized by findings that elevated levels of PPAR-γ promote the polarization of monocyte-derived macrophages toward the anti-inflammatory M2 phenotype in the lungs, initiating resolution of inflammation [184].

However, deficiency in the levels of PPAR-γ promotes the polarization toward the pro-inflammatory M1 phenotype. Conversely, PPAR-γ activation suppresses the transcription of various pro-inflammatory genes and reduces the production of inflammatory mediators [185]. Deficiency in PPAR-γ leads to an exaggerated pulmonary inflammatory response and impaired resolution of inflammation [186]. This polarization is regulated in part by microRNAs that modulate PPAR-responsive gene expression [187]. PPAR-γ also upregulates CD36 expression, thereby, enhancing the phagocytosis of apoptotic neutrophils by macrophages, a process which contributes to the resolution of inflammation [188, 189].

The role of PPARs in pulmonary immune regulation was also supported by studies showing reduced expression of especially PPAR-γ and associated microRNAs in the lungs of COVID-19 patients and in macrophages from patients with influenza infection, correlating with increased production of pro-inflammatory mediators and greater severity of respiratory failure [32, 185, 190, 191]. Lung biopsies from COVID-19 patients revealed suppressed PPAR-γ expression in M1 macrophages, which also correlated with disease severity [32]. ANG II inhibits PPAR-γ transcription, providing a mechanistic explanation for the decreased PPAR-γ levels observed in ssRNA virus infections, contributing to the polarization toward the pro-inflammatory M1 phenotype and a persistent inflammatory response [192].

The impact of CD40L-CD40 activation on the brain

Microglia, the resident macrophages of the central nervous system (CNS) play a key role in regulating inflammatory responses in the brain. Under normal conditions, CD40 expression on microglia is low. However, during inflammatory responses—such as those triggered by respiratory ssRNA virus infections—the release of interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) significantly upregulates CD40 expression on microglial cells over time [193].

Microglia expressing CD40 are highly responsive to CD40L, either in its membrane-bound form by brain-infiltrating activated $CD4^+$ T cells or as soluble CD40L. This interaction triggers microglia to release pro-inflammatory mediators in the brain, particularly chemokines [165, 194]. Patients with severe COVID-19 exhibit increased $CD4^+$ T cell activity and elevated levels of soluble CD40L, both of which can contribute to CD40L/CD40-mediated microglial activation [84]. Activation of the CD40/CD40L signaling pathway in the CNS has been shown to increase blood–brain barrier permeability, promote edema, damage glial cells, and facilitate microthrombi formation [195]. Furthermore, this microglial activation initiates intracellular signaling cascades that drive the production of cytokines, chemokines, and neurotoxins, thereby amplifying pro-inflammatory responses within the CNS [194]. These neuroinflammatory processes have been closely associated with cognitive dysfunction, including "brain fog," fatigue, and other nonspecific cerebral symptoms commonly observed in intensive care unit (ICU) patients with

COVID-19, as well as in individuals suffering from long COVID [196, 197]. Thus, CD40 expression on microglia appears to play a pivotal role in mediating virus-independent inflammatory responses and neurological sequelae in CNS.

TLR 8 activates the cathelicidin/vitamin D pathway

In addition, TLR8 activation by ssRNA viruses upregulates the enzyme CYP27B1. It converts vitamin D from its inactive form (calcifediol) to the bioactive form (calcitriol). Calcitriol binds to the vitamin D receptor (VDR), which translocates into the nucleus and functions as a transcription factor to induce the production of the antimicrobial peptide cathelicidin [198]. Cathelicidin has been shown to bind and induce autophagy of viral antigens, contributing to clearance of several ssRNA viruses, such as SARS-CoV-2, rhinovirus, RSV, and influenza virus [199-202].

Healthy individuals, who are exposed to sunlight and have a mixed adequate nutritional vitamin D intake have usually normal D-vitamin levels. However, during ssRNA virus infections a gradual and insidious decline in vitamin D levels occurs, due to the upregulation of CYP27B1 and consumption of existing calcifediol. Long lasting or chronic infections may therefore cause vitamin D deficiency. It may then impair the production of cathelicidin, which subsequently prolongs the elimination of ssRNA viruses and delay recovery. Low vitamin D levels are associated with increased severity and mortality in SARS-CoV-2, influenza and RSV infections [203-205]. Low serum levels of vitamin 25(OH)D were the only variable significantly associated with long COVID in a multivariate analysis [206]. Vitamin D deficiency are also linked to multiple sclerosis (MS), a chronic inflammatory condition believed to have a post-infectious etiology related to viral exposure [207]. Vitamin D deficiency is also associated with other viral infections and autoimmune diseases [206, 208]. As part of the continuous debate regarding D-vitamin intake, it seems important to maintain a normal D-vitamin level and being prepared to boost the intake during the acute course of a virus infection [209, 210]. Since CYP27B1 and the activation of vitamin D occur when the ssRNA virus stimulates TLR8 in the endosomes of the primary infected cells, it is not certain that vitamin D supplementation are of major importance later on when the infection has been established or becoming long lasting.

"The reference list can be found in Part Two of this divided narrative review."