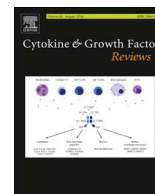




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# The cytokine storm in COVID-19: An overview of the involvement of the chemokine/chemokine-receptor system



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## ABSTRACT

In 2019–2020 a new coronavirus named SARS-CoV-2 was identified as the causative agent of a severe acute respiratory infection named COVID-19, which is causing a worldwide pandemic. There are still many unresolved questions regarding the pathogenesis of this disease and especially the reasons underlying the extremely different clinical course, ranging from asymptomatic forms to severe manifestations, including the Acute Respiratory Distress Syndrome (ARDS). SARS-CoV-2 showed phylogenetic similarities to both SARS-CoV and MERS-CoV viruses, and some of the clinical features are shared between COVID-19 and previously identified beta-coronavirus infections. Available evidence indicate that the so called “cytokine storm” an uncontrolled over-production of soluble markers of inflammation which, in turn, sustain an aberrant systemic inflammatory response, is a major responsible for the occurrence of ARDS. Chemokines are low molecular weight proteins with powerful chemoattractant activity which play a role in the immune cell recruitment during inflammation. This review will be aimed at providing an overview of the current knowledge on the involvement of the chemokine/chemokine-receptor system in the cytokine storm related to SARS-CoV-2 infection. Basic and clinical evidences obtained from previous SARS and MERS epidemics and available data from COVID-19 will be taken into account.

## 1. Coronavirus: the past and the present

Coronaviruses, a genus of the *Coronaviridae* family, are enveloped viruses with a large plus-strand Ribonucleic Acid (RNA) genome [1]. The genomic RNA is 27–32 kb in size, capped and polyadenylated. Coronaviruses were identified in several non-human species, including rats, mice, chickens, cattle, turkeys, swine, cats, dogs, rabbits and horses. In these species, Coronavirus infection often causes devastating epizootics of respiratory or enteric diseases. Several coronaviruses, such as HCoV-229E and HCoV-OC43, were identified since the mid-1960s. Prior to the SARS-CoV outbreak, coronaviruses were only thought to cause mild, self-limiting respiratory infections in humans, commonly referred to as “colds”. These viruses are endemic among the human populations, causing 15–30 % of respiratory tract infections each year. Rarely, these viruses can cause lower respiratory tract infections,

especially in neonates, in the elderly, and in individuals with underlying illnesses.

SARS-CoV, a novel coronavirus, was identified in 2002 as the pathogenic agent of the Severe Acute Respiratory Syndrome (SARS) outbreak that occurred in the Guangdong Province of China [2]. So far, SARS is the most severe human disease caused by a coronavirus. Recent evidence confirmed that the SARS-CoV virus originated from a mutation occurring in a non-human host, probably bats, gains the ability to affect humans. Luckily, the transmission of SARS-CoV was relatively inefficient, since its spread occurred only through direct contact with infected individuals, with negligible infectivity during incubation state. The outbreak was largely contained within households and healthcare settings.

A novel human Coronavirus, named Middle East Respiratory Syndrome-CoV (MERS-CoV), emerged in the Middle East in 2012 as the

**Abbreviations:** AP-1, activator protein-1; ARDS, acute respiratory distress syndrome; COVID-19, Coronavirus Disease 2019; CSF, colony-stimulating factors; DNA, deoxyribonucleic acid; ICU, Intensive Care Units; IFN, interferon; IL-, interleukin; IRF, interferon regulatory transcription factor; LPS, lipopolysaccharide; MERS, Middle East Respiratory Syndrome; MERS-CoV, Middle East Respiratory Syndrome Coronavirus; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PBMCs, Peripheral Blood Mononuclear Cells; RNA, Ribonucleic Acid; SARS, Severe Acute Respiratory Syndrome; SARS-CoV, Severe Acute Respiratory Syndrome Coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TH1, T-helper-1; TNF, Tumor Necrosis Factor

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causative agent of a series of highly pathogenic respiratory tract infections in Saudi Arabia and other countries in the Middle East [3]. In the early stages of the outbreak, a high mortality rate of ~50 % was reported, but the outbreak did not accelerate through 2013 and by the end of 2014 it was largely controlled. Also for this virus, a zoonotic origin is suspected, since dromedary camels may be its natural hosts.

In early December 2019, several pneumonia cases of unknown origin were observed in Wuhan (China). The pathogen was identified as a novel enveloped RNA  $\beta$  coronavirus that was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [4]. The virus showed phylogenetic similarities to both SARS-CoV and MERS-CoV viruses. In view of its similarities to bat coronaviruses, it was postulated that bats could have been the primary hosts of SARS-CoV-2. This hypothesis suggested that the infection originated *via* transmission from wild animals illegally sold in the Huanan Seafood Wholesale Market. On January, 30th, 2020, The World Health Organization (WHO) declared coronavirus disease 2019 (COVID-19) a public health emergency of international concern, and on March 11th, WHO Director General referred to COVID-19 as a pandemic. As of May 5th, 2020, the number of confirmed cases of COVID-19 has exceeded 3 million worldwide, with more than 250,000 COVID-19-related deaths. The epidemic has put public health systems under severe strain both in western countries and in the developing world. SARS-CoV-2 displays a more efficient transmission pattern when compared with SARS-CoV and MERS-CoV [5], retaining a high transmission rate also in the asymptomatic incubation period [6]. The clinical spectrum of COVID-19 syndrome varies remarkably, going from asymptomatic forms to acute bilateral pneumonias requiring hospitalization. Common presenting symptoms include fever, fatigue and dry cough, while laboratory tests often show lymphopenia and elevated lactate dehydrogenase levels. Chest computed tomographic scans show a typical pattern of bilateral patchy shadows or ground glass opacity. A significant percentage of cases requires admission to intensive-care-units (ICU) due to acute respiratory distress syndrome that requires mechanical ventilation support. A subgroup of patients with severe COVID-19 can experience the so-called “cytokine storm syndrome”, characterized by a fulminant and fatal hyper-cytokemia associated with multi-organ failure.

## 2. The cytokine storm induced by SARS-Cov-2 infection

The term “cytokine storm” has become increasingly used not only by Authors of scientific articles but also by popular media. It is likely that the widespread use of this term is related with its rather immediate meaning, which actually recalls the role of the immune system in producing an uncontrolled and generalized inflammatory response [7]. It seems not casual that the term cytokine storm was first employed in describing the events modulating the onset of the graft-versus-host disease [8], a condition characterized by an impressively powerful activation of the immune system. Cytokine storms characterize a wide spectrum of infectious and non-infectious diseases, and since 2005, it was associated to the avian H5N1 influenza virus infection [9]. Apart from the immediate significance of the term cytokine storm, the biological and clinical consequences of this immune system hyperactivity are by far less known, making it worthwhile to be briefly overviewed.

There are several similarities in the clinical features between COVID-19 and previously identified beta-coronavirus infections. Shared clinical findings include that most patients present with fever, dry cough, dyspnea, and bilateral ground-glass opacities on chest CT scans [10]. However, the physiopathology of the mechanisms through which SARS-CoV or MERS-CoV sustain high pathogenicity are yet to be completely unveiled.

Since the first reports on COVID-19 disease, it appeared clear that Acute respiratory distress syndrome (ARDS) accounted for a significant number of deaths among infected patients and that ARDS should be regarded as the hallmark immune-mediated clinical consequence in SARS-CoV-2, similarly to what described for SARS-CoV and MERS-CoV

infections [11]. Acute respiratory distress syndrome (ARDS) is a devastating event, with an estimated mortality of approximately 40 %, defined as the presence of bilateral lung infiltrates and severe hypoxemia. ARDS can occur in a variety of clinical situations, including pneumonia, sepsis, pancreatitis, blood transfusion. ARDS pathogenesis involves inflammatory injury to the alveolo–capillary membrane, which results in increased lung permeability and the exudation of protein-rich pulmonary edema fluid into the airspaces, leading in the end to respiratory insufficiency [12].

As shown by previous data in the literature, increased circulating levels of pro-inflammatory cytokines (eg, Interferon  $\gamma$ , interleukin (IL)-1B, IL-6, IL-12) and chemokines (CXCL10, and CCL2) are associated with pulmonary inflammation and extensive lung involvement in SARS patients, similarly to what happens in MERS-CoV infection [13]. As far as COVID 19 infection is concerned, Huang et al. recently reported that infected patients also show high levels of pro-inflammatory cytokines and chemokines [4]. The demonstration of increased levels of IL-1B, IFN $\gamma$ , CXCL10, and CCL2 strongly pointed toward an activation of T-helper-1 (Th1) cell function. More importantly, the so called “cytokine storm” emerged as a main factor driving a more severe clinical course. This concept originated from the observation that COVID-19 patients requiring ICU admission displayed higher concentrations of CXCL10, CCL2 and TNF $\alpha$  as compared to those in which the infection was less severe and did not require an ICU admission. To further complicate the issue, it should be highlighted that, in patients with SARS-Cov-2 infection, at difference from SARS-CoV infection, there is also an increased secretion Th2-immune-oriented cytokines such as IL-4 and IL-10, whose main effect is to suppress inflammation [14].

Taken together, these data clearly indicate that, in SARS-CoV infection, ARDS is the ultimate result of a cytokine storm. In this scenario, the release by immune effector cells of large amounts of pro-inflammatory cytokines (IFN $\alpha$ , IFN $\gamma$ , IL-1 $\beta$ , IL-6, IL-12, IL-18, IL-33, TNF $\alpha$ , TGF $\beta$ ) and chemokines (CXCL10, CXCL8, CXCL9, CCL2, CCL3, CCL5) precipitates and sustains the aberrant systemic inflammatory response [4,13,15,16]. The cytokine storm is readily followed by the immune system “attacking” the body, which in turn will cause ARDS and multiple organ failure, the final result being death, at least in the most severe cases of SARS-CoV-2 infection [11].

The cytokine storm, and the consequent ARDS, results from the effects of a combination of many immune-active molecules. Interferons, interleukins, chemokines, Colony-stimulating factors and TNF-alpha represent the main components involved in the development of the Cytokine storm and will be briefly overviewed.

- **Interferons**, a family of cytokines with a central role in virus-directed innate immunity binds specific receptors and result in the expression of genes encoding protein with anti-viral or immunomodulatory properties. This sequence of events supported the therapeutic use of IFNs in some viral diseases such as chronic hepatitis, but also in non-viral conditions including leukemia and lymphoma, melanoma and multiple sclerosis [17,18].

- **Tumor necrosis factor  $\alpha$  (TNF $\alpha$ )** is a pyrogen cytokine released from immune cells in the acute phase of inflammation and infection. It is a central cytokine in viral diseases and is associated with a number of chronic inflammatory and autoimmune diseases [19].

- **Colony-stimulating factors (CSF)**. These proteins are associated with inflammatory conditions and are components of an amplification cascade which ultimately increases cytokine production by macrophages at sites of inflammation, This effect perpetuates the inflammatory reaction [20].

- **Interleukins** are a family of cytokines involved in immune cells differentiation and activation. They mediate the traffic of immune cells to the site of the infection, induce the increase of the acute phase signaling, activate epithelial cells and mediate the production of secondary cytokines [21]. Among them, Interleukin-6 (IL-6) deserves a more extensive discussion in view of its involvement in the coronavirus-induced cytokine storm. IL-6 is crucially involved in acute

inflammation due to its role in regulating the acute phase response [21]. It is produced by almost all stromal cells and B lymphocytes, T lymphocytes, macrophages, monocytes, dendritic cells, mast cells and other non-lymphocytic cells, such as fibroblasts, endothelial cells, keratinocytes, glomerular Mesangial cells and tumor cells [22]. The production of this cytokine is increased by IL-1 $\beta$  and tumor necrosis factor (TNF- $\alpha$ ) [23]. IL-6 may also be responsible for the activation of T helper 17 (TH17) cells in the dendritic cell-T cell interaction [24]. In COVID-19 affected patients, a high TH17 cells activation could result from a virus-driven increased production of IL-6 by the immune system. IL-6 plays a key role in the pathogenesis of the cytokine storm owing to its pleiotropic properties. Several studies showed that the serum levels of IL-6 are increased in COVID-19 patients and that its circulating levels are positively related to disease severity [14,25,26]. For this reason, high serum IL-6 levels were suggested as predictors for disease severity [27,28]. Indeed, in animal models of SARS-CoV infection, the inhibition of the transcription factor of IL-6 and, in turn of its production, was associated with reduced mortality [29].

During the present COVID-19 pandemic, the use of Tocilizumab as a therapeutic agent was proposed. Tocilizumab is a humanized anti-IL-6 receptor IgG1 monoclonal antibody used for the treatment of rheumatoid arthritis and other chronic inflammatory diseases [14]. By blocking the IL-6-receptor interaction, Tocilizumab inhibits the IL-6-mediated signal transduction. Although clinical data on the use of Tocilizumab in COVID-19 patients derive from small series, some authors recommend its use in critically ill COVID-19 patients with significantly elevated IL-6 levels [14].

**-Chemokines** are a large family of cytokines characterized by a powerful chemotactic effect. Chemokines

act as chemo-attractants in the migration of the immune system cells, but they are also involved in several other processes including the development and function of innate and adaptive immune system, embryogenesis, and cancer metastasis [30,31]. They are promptly secreted by a variety of cells in response to viral or microbial infections [32].

Chemokines act as powerful chemoattractants which recruit inflammatory cells to migrate from the intravascular space across the endothelium and epithelium into the inflammation site, according to a chemokine gradient [33]. The role of one specific chemokine, CXCL10 (previously referred to as interferon- $\gamma$  inducible protein of 10 kDa, or IP-10), has been highlighted in ARDS in both experimental models and in patients.

Indeed, in a mouse model of IL-2 –induced ARDS, an up-regulation of the mouse CXCL10 analogue mob-1 mRNA was observed at initiation of lung injury [34]. Several studies also showed that the intratracheal injection of mob-1 in mice induced pulmonary migration of leukocytes in the alveolar space, with massive recruitment of neutrophils, especially monocytes. This event was rapidly followed by microvascular injury and pulmonary edema typical of ARDS [35,36]. CXCL10 signaling appears to be a critical factor for the onset of ARDS, as shown in mice models of ARDS induced by either acid aspiration or by viral infection (with influenza H5N1 virus). Briefly, Ichikawa et al., demonstrated that wild-type mice developing ARDS had increased levels of CXCL10 mainly due to an increased secretion by infiltrating neutrophils, which induced an autocrine loop mechanism on the chemotaxis of inflamed neutrophils, leading to fulminant pulmonary inflammation. On the contrary, CXCL10 and/or its receptor CXCR3 knock-out mice showed decreased lung injury severity and increased survival in response to both viral and non-viral lung injury [37]. Moreover, CXCL10 expression in the lung was significantly up-regulated after induction of ARDS with Lipopolysaccharide (LPS) in a mouse model of lung injury, and the neutralization of CXCL10 with anti-CXCL10 antibody lead to amelioration of lung injury [38].

CXCL8 (also referred to as IL-8) is another chemokine considered as a potential prognostic bio-marker for ARDS clinical course [39]. Indeed, CXCL8 levels were found to be elevated both in plasma [40–43] and in

the broncho-alveolar lavage fluid [44–46] of patients with ARDS. A direct role of CXCL8 in the progression of ARDS was proven in rabbit with acid-induced ARDS lead to a 10-fold increase in CXCL8 levels in the alveolar fluids. Of note, pre-treatment with an anti-CXCL8 antibody prevented the development of the typical acute lung injury [47].

Although chemokines are crucially involved in the regulation and maintenance of immune responses, their role in the onset of the coronavirus-induced cytokine storm is still poorly investigated.

### 3. Chemokines: small molecules with an important role in inflammatory diseases

Chemokines are a family of low molecular weight proteins expressed, both constitutively and in an inducible manner, by several types of cells. Chemokines play an important role in the inflammatory response by attracting leukocytes to sites of infection. These small proteins also contribute to the homeostatic circulation of leukocytes through tissues [31]. At present, 50 chemokines and 20 chemokine receptors have been recognized and classified. Chemokines are named according to the most recent nomenclature, which classifies them according to their chemical structure, the C, CC CXC and CX3C families [48]. The binding of chemokines to their receptors is responsible for their chemoattractant ability. The chemokine receptors belong to the seven-transmembrane-spanning, G-protein-coupled receptors, which are expressed primarily on leukocytes but also on other cells, e.g., endothelial cells [49]. The many functions of chemokines include the control of cell proliferation and differentiation, the regulation of angiogenesis and immune and inflammatory responses, tumor growth and metastasis [50–52]. Most recently, several studies investigated the involvement of chemokines in coronavirus-related infective disease. It emerged that specific chemokines could play a crucial role in the development of COVID-19-related symptoms, thus confirming what previously known for other types of coronaviruses, such as SARS and MERS. [13,53]. These findings could be somehow expected in view of the well-known role of chemokines in viral infections.

#### 3.1. The involvement of chemokines in viral infections

Before addressing the specific relationship between chemokines and coronavirus infections, it is mandatory to briefly overview the general role of chemokines in viral infections and how viruses contrast the actions of chemokines.

Viruses are infectious agents of small size and simple composition that can multiply only in living cells of animals, plants, or bacteria. All viruses contain a nucleic acid, either -DNA (deoxyribonucleic acid) or -RNA (ribonucleic acid), and several proteins. Viruses should not even be considered organisms since they are not free-living (i.e., they require a host cell), thus viruses need to elude the host immune defense to infect its cells in order to reproduce and survive. [54].

The chemokine/chemokine receptor-related immune defenses are the main obstacles to be by-passed by viruses. Some chemokines play a direct anti-viral effect by inducing an array of phenomena that lead cells to determine an “anti-viral” state. These phenomena include activation of apoptosis or direct killing of infected cells by activated immune cells. Chemokines also recruit immune cells to the site of infection, which will fight against the intruder [55]. Viral infections are associated with enhanced expression of several chemokines, in particular the interferons-inducible ones. Interferons, which can be produced by any mammalian cell, are involved in the rapid and efficient host innate response against viruses. A powerful IFN response triggered by the first contact with a virus can slow down viral multiplication and “buy time” for the organism to establish a more efficient adaptive immune response [56]. IFNs can stimulate surrounding cells to express potent antiviral proteins including enzymes, transcription factors, cell surface glycoproteins, cytokines and chemokines [57,58]. Moreover, they can inhibit cell proliferation, regulate apoptosis and modulate the

immune response [59]. Among interferons-induced molecules, the chemokine CXCL10 is currently regarded as a main player in the organism anti-viral response [60], and particularly in respiratory tract infections. Several studies demonstrated that CXCL10 levels, as evaluated in serum, bronchial-alveolar washing fluid or nasal secretions, consistently correlate with the severity and duration of acute respiratory tract infection due to viral infections [60–62].

Also the chemokine CXCL8, is involved in inflammation and immune cell trafficking in the context of viral infections. CXCL8 plays a major role in the initial control of respiratory tract infection due to its chemotactic activity for neutrophils and monocytes [63]. CXCL8 levels in the nasal washing fluid correlate with symptoms severity during acute respiratory tract infections [64]. Although in the majority of cases a strong chemokine action can efficiently contrast viral infections, some viruses acquire the capacity of escaping this surveillance system. Furthermore, viruses can use the chemokine system network for their own favor by several strategies:

- Some viruses “mimic” the components of the chemokine system by producing molecules that are very similar to chemokines and can interact with their receptor. These molecules generate an incongruous signal leading to a disorganized immune response to viruses [65].
- Inhibition of the interferon-induced anti-viral response. Several viruses do impair the intracellular receptors devoted to pathogen recognition, such as Toll like receptors and intracellular RNA sensors. [66]

Taken together, the above data indicate that viruses can interfere with the chemokine/chemokine-receptors system using their own properties to modify intracellular signaling with the final result to further disseminate the infection.

#### 4. Chemokines and Corona Virus: Lessons from the SARS and the MERS epidemics and available data from COVID-19

The strict relationship between Coronaviruses infection and chemokines has been thoroughly investigated during both the SARS-CoV and the MERS-CoV epidemics, while some initial data are available regarding SARS-CoV-2 and its related syndrome, COVID-19.

##### 4.1. SARS-CoV

Since the first reports of SARS, it seemed clear that the severe clinical manifestations of the disease could not be ascribed only to the viral activity *per se*, but that an immune-mediated mechanism rather than a direct virus-induced damage would drive the clinical progression [67]. Indeed from the physio-pathology point of view, the most interesting observation was the demonstration that viral titers seemed to paradoxically diminish during the most severe phase of the disease both in humans and in several animal models [1]. Data coming from patients’ series described during the 2003–2004 epidemics clearly suggested that complex alterations in the chemokine system were related to the outcome of SARS-CoV infection (Fig. 1).

*In vivo* studies showed that several circulating chemokines (CXCL8, CCL2 and CXCL10) and inflammatory cytokines (IL-1, IL-6 and IL-12) were elevated in patients with SARS-CoV [68,69]. CXCL10 was also considered an excellent prognostic marker for SARS disease progression [70,71]. In particular, Jiang et al. showed that CXCL 10 serum levels were significantly increased during the early stage of SARS, and remained elevated until resolution. Moreover, persistently elevated CXCL10 serum levels during follow-up were predictive of a worse outcome of the infection [71]

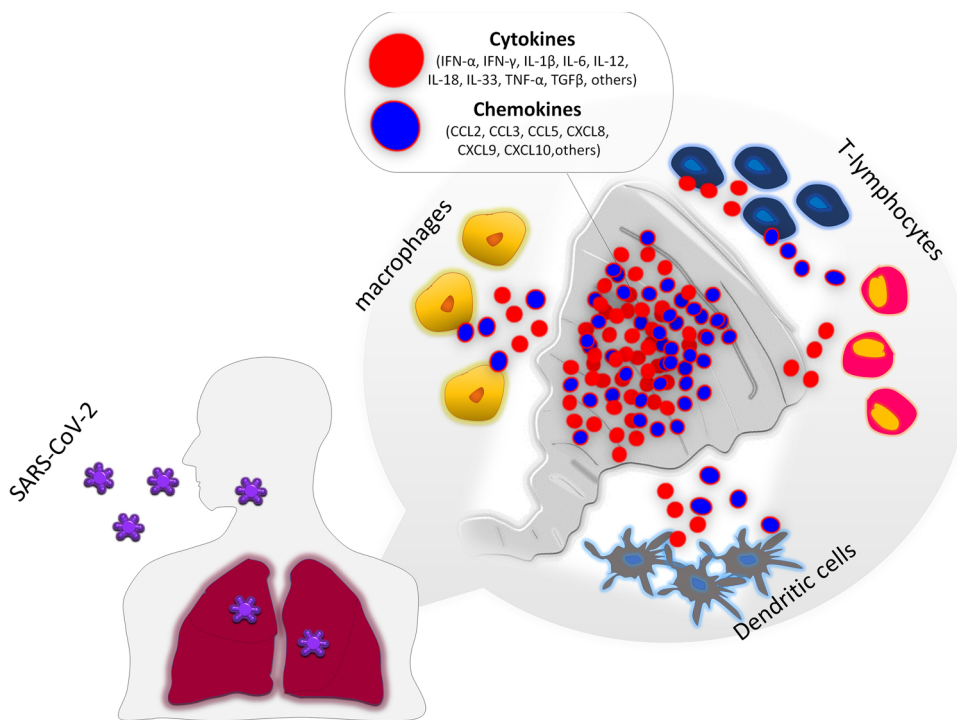
These findings prompted further *in vitro* studies aimed at investigating the relationship between SARS and the chemokine system. Spiegel et al., demonstrated that, in addition to its direct effect on epithelial lung cells, SARS-CoV could also enter macrophages and dendritic cells [72]. This appeared crucial as viral entrance in these cells lead to an abortive infection (e.g. the virus enters the host-cell but cannot successfully complete replication). Yet the virus elicited the secretion of pro-inflammatory chemokines by dendritic and macrophages cells [73]. This finding was confirmed *in vivo* because the serum levels of a wide spectrum of cytokines and chemokines produced by dendritic cells and macrophages were elevated in SARS-CoV infected patients [74]. Furthermore, the infection with SARS-CoV of human primary myeloid-derived dendritic cells was followed by an impaired defensive IFN $\beta$  response, which was paralleled by a moderate up-regulation of pro inflammatory cytokines (such as TNF- $\alpha$  and IL-6) and a much more significant up-regulation of inflammatory chemokines (such as CXCL10, CCL3, CCL5, and CCL2) [75]. The authors suggested that the lack of response to antiviral interferons in the presence of chemokine up-regulation could represent a further mechanism of immune evasion by SARS-CoV [73]. In line with this hypothesis, the direct exposure of lung epithelial cells [76] or Peripheral Blood Mononuclear Cells (PBMCs) [75] coming from SARS infected patients to viral proteins (such as S-protein and N-protein) induced a prompt release of several chemokines, including CXCL8 and CXCL10. *In vitro* gene-expression studies also reported that PBMC from normal healthy donors inoculated with SARS-CoV showed an early enhancement in the expression of several chemokines belonging both to the CC family (CCL4, CCL20, CCL22, CCL25, CCL27, and their receptors CCR4, CCR7) and of the CXCL family (CXCL8 and IL-17) [77]. Additional data came from animal models of SARS-CoV infection. In mice infected with SARS-CoV, the clinical features of the syndrome showed an age-dependent increase in severity (similarly to what observed in humans), which was related to an increased level of pro-inflammatory cytokines and chemokines, paralleled by a reduction in T-cell responses [78]. Another study showed that in mice infected with SARS-CoV, robust virus replication accompanied by delayed type I interferon secretion caused a rapidly fatal pneumonia. This delayed Type I-interferon signaling promoted the accumulation of pathogenic inflammatory monocyte-macrophages, with resulting increase in cytokine (IL-6) and chemokine (CCL2) lung levels, vascular leakage, and impaired virus-specific T cell responses. [79].

These data suggest that coronaviruses, and in particular SARS-CoV, have a peculiar ability to counteract the antiviral IFN response, pointing toward the fact that the severity of disease might be due to immune dysregulation, rather than to the level of viremia. This dysregulation would be characterized by an insufficient type I interferon response (too little and too late), paralleled by an aberrant pro-inflammatory chemokine secretion by alveolar macrophages, dendritic cells and pneumocytes [66,80].

*In vitro* data suggest that this class of viruses, and in particular SARS-CoV, uses several strategies to avoid Type I IFN response, both passive and active [56,59].

- Passive mechanisms include the induction of double membrane vesicles (DMV) at perinuclear sites within the cytoplasm where RNA synthesis takes place. This strategy may help to hide and protect RNA replication intermediates from being sensed by intracellular RNA-sensors, thus avoiding the activation of the IFN cascade [81,82].
- Active mechanisms include a direct action of viral proteins on transcription factors and intracellular signaling molecules that regulate the IFN cascade. In particular, the SARS-CoV protein ORF6 is able to inhibit the action of interferon regulatory transcription





**Fig. 1.** Schematic representation of the “Cytokine storm” development after SARS-CoV-2 infection. The presence of SARS-CoV-2 in the lung induces an uncontrolled generalized immune response. Several immune cells (like T-lymphocytes, macrophages and dendritic cells) sustain the impressive secretion of cytokines and chemokines ultimately leading to acute respiratory distress syndrome.

factor-3 (IRF-3), a transcription factor of the IFN genes [83,84].

The fact that SARS-CoV infection would upregulate the transcription of CXCL10, while significantly down-regulating IFNs signaling, could seem paradoxical. However, transcriptional enhancement of CXCL10 could be due to a direct effect on the Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) [85] triggered by SARS-CoV [86], even if other Authors did not confirm this early observation [87]. Similarly, the up-regulation of the CXCL8 gene expression, could be due to a direct effect of the virus at the cellular level. Indeed, intestinal and lung cells lines infected by SARS-CoV, promptly increase their secretion of CXCL8 [88]. This observation would fit with the notion that the expression of CXCL8 is dependent on the transcription factor Activator protein 1 (AP-1), which was shown to be strongly up-regulated by SARS-CoV [86,89].

#### 4.2. MERS-CoV

During the MERS-CoV epidemics, several studies were aimed at understanding the pathogenic mechanisms underlying the severe and often fatal pneumonia were performed. Available data suggest that MERS-CoV infection shares some immunological aspects of SARS-CoV infection, in terms of involvement of the chemokine system. First, an increase in the serum levels of CXCL10 when compared to controls was observed also in MERS-CoV patients. More importantly, a persistent CXCL10 increase was associated with disease severity [90].

In this regard, the case of two paradigmatic patients diagnosed with MERS-CoV is worth noting. One had a fatal outcome and experienced an impaired IFN response together with a relevant increase in serum CXCL10 levels. The other one, with a favorable outcome, displayed an up-regulation of IFNs and IRF3 and a less pronounced increase of serum CXCL10 levels [91].

*In vitro* studies also support the relevance of chemokines in MERS-CoV patients. A 2013 study evaluated the expression of several chemokines and cytokines in cell lysates of polarized airway epithelial cells infected with MERS-CoV or SARS-CoV. The results showed that CXCL8

was up-regulated to a greater extent by MERS-CoV infection as compared with SARS-CoV. At difference, CCL2 and CXCL10 were more strongly up-regulated by SARS-CoV than MERS-CoV [92]. In addition, in dendritic cells infected with MERS-CoV, a significant down-regulation of IFN response paralleled by a striking elevation of CXCL10 was observed [93]. A strong induction of CXCL10 secretion was observed also in monocyte-derived macrophages infected with MERS-CoV [94,95]. Interesting data come also from an experimental mouse model of MERS-CoV infection, in which a significant increase in CXCL8 expression was observed in lung and brain tissue after infection with MERS-CoV [96].

#### 4.3. SARS-CoV-2 and COVID-19 syndrome

Data regarding the relationship between COVID-19 and chemokine dysregulation are still scanty, but are increasingly being reported. Although preliminary, the available data provided by both clinical and *in vitro* studies suggest possible similarities between what was observed after SARS-CoV and MERS-CoV infection. Overall, an increased production of pro-inflammatory chemokines (mainly CXCL10 and CXCL8) seems to characterize also COVID-19 infection.

There are few *in vitro* studies regarding SARS-CoV-2 infection published so far. The results of a study comparing SARS-CoV-2 and SARS-CoV viruses behavior in the lung tissue should be overviewed. Briefly, the inoculation of the two viruses in *ex vivo* human lung tissue explants showed that SARS-CoV-2 was more capable, as compared with SARS-CoV, in both infecting and replicating in human lung. Furthermore, SARS-CoV-2 infection was less able to trigger the expression of any IFNs, suggesting that SARS-CoV and SARS-CoV-2 might differ in their ability to modulate the production of pro-inflammatory cytokines and chemokines. As an example, it could be worth highlighting that SARS-CoV infection upregulated 11 out of the 13 pro-inflammatory factors evaluated, while SARS-CoV-2 upregulated only five of them, (namely, CXCL10, IL6, CCL2, CXCL1, CXCL5). Interestingly, CXCL8 transcription was up-regulated only by SARS-CoV, but not SARS-CoV-2 infection, while the opposite was observed for CXCL10 [97].

The potential involvement of the chemokine system during SARS-CoV-2 infection was already evident from the first COVID-19 series described by Chinese physicians in early January 2020. It was reported that several pro-inflammatory cytokines and chemokines, including CXCL10, CXCL8, CCL2, TNF $\alpha$  and IFN $\gamma$  were higher in the plasma of COVID-19 patients as compared to healthy controls. More importantly, among infected patients, CXCL10, CCL2 and TNF $\alpha$  circulating concentrations (but not those of IFN $\gamma$ ) were found to be significantly higher in patients requiring admission to Intensive Care Units as compared to patients experiencing a less severe clinical course [4].

Chen et al., recently characterized the immunological features of COVID-19 patients with different severity of the disease. Briefly, the 11 patients with severe disease displayed significantly higher serum levels of IL-6, IL-10, and TNF- $\alpha$  and lower absolute numbers of T lymphocytes, CD4 + T cells, and CD8 + T cells as compared with the 10 patients with moderate disease. Of note, severe cases were characterized by a lower expression of IFN- $\gamma$  by CD4 + T cells as compared with moderate cases. [98]

Xiong et al. performed a transcriptome sequencing analysis of several pro-inflammatory genes in both PMCs and broncho-alveolar lavage fluid of patients with COVID-19, compared with samples from healthy donors. The authors reported that COVID-19 patients showed up-regulation of genes encoding for several immune-regulatory molecules, in particular CXCL10, in PBMCs whereas no up-regulation of CXCL10 gene was observed in broncho-alveolar lavage fluid. Moreover, an up-regulation of several genes involved in apoptosis and P53 signaling pathways in PBMC was observed, leading to the hypothesis that this process could be the underlying cause of lymphopenia commonly observed in COVID-19 patients. [99]. Another study by Wang et al., including 65 SARS-CoV-2-positive patients showed that the absolute numbers of CD4 + T cells, CD8 + T cells and B cells progressively decreased in relation with increasing severity of illness. [100]. In this view, the findings recently reported by Yang et al. appear of potential clinical relevance. Indeed, several cytokine/chemokine levels were measured and found to be elevated in patients with different clinical severity of the COVID-19. Among them, CXCL10, CCL7 and IL-1 receptor antagonist were the ones significantly related to disease severity and even more importantly, CXCL10 levels were the only one to be positively and significantly correlated with the viral load (Yang, et al. 2020).

## 5. Conclusive remarks

SARS-CoV-2, and its related syndrome COVID-19, have been known to the scientific community since less than 5 months. Clearly much is yet to be understood, and the challenge for the next future will be to increase our understanding the physiopathology of this novel infectious disease. Hopefully, the advances in our comprehension of the mechanisms sustaining the clinical course and patients-related factors driving the final outcome will be helpful in developing effective preventive strategies and/or therapeutical options. Based on current knowledge, the “cytokine storm” appears as one of the most dangerous and potentially life-threatening event related to COVID-19 sustaining its major clinical consequences. The immune mediated events related to the response to SARS-CoV-2 infection, and the role of the chemokine/chemokine receptor system, will be further and more extensively characterized with the final goal to identify targeted therapeutic strategies. Although lessons from the previous SARS and MERS epidemics can be drawn, there is still much to do in order to conclude whether SARS-CoV-2 virus behaves in the same way of its predecessors or if it is characterized by peculiar specificities. Clearly, the hide-and-seek challenge between the virus and our immune defenses will also help us understanding the extremely variable spectrum of clinical manifestations of COVID-19, which appears to range between asymptomatic cases to possibly lethal bilateral pneumonia with multi-organ failure.

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## CRediT authorship contribution statement

**Francesca Coperchini:** Conceptualization, Methodology, Writing - original draft. **Luca Chiovato:** Supervision. **Laura Croce:** Writing - original draft. **Flavia Magri:** Writing - review & editing. **Mario Rotondi:** Conceptualization, Writing - review & editing, Supervision.

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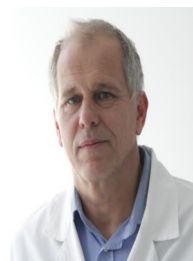
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