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VIEWPOINT

Blocking inflammation on the way: Rationale for CXCR2 antagonists for the treatment of COVID-19

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An exacerbated and unbalanced immune response may account for the severity of COVID-19, the disease caused by the novel severe acute respiratory syndrome (SARS) coronavirus 2, SARS-CoV-2. In this Viewpoint, we summarize recent evidence for the role of neutrophils in the pathogenesis of COVID-19 and propose CXCR2 inhibition as a promising treatment option to block neutrophil recruitment and activation.

The pathomechanism leading to life-threatening courses in COVID-19, the disease caused by the recently identified severe acute respiratory syndrome (SARS) coronavirus 2, SARS-CoV-2, is not fully understood. While the viral load decreases in later stages of COVID-19, symptoms frequently do not resolve concomitantly. It is widely appreciated that an overreactive immune system with infiltration of inflammatory monocytes and neutrophils to the site of infection alongside an exaggerated release of proinflammatory cytokines is an important driver of severe lung damage in COVID-19 (Vabret et al., 2020). In some cases, this leads to acute respiratory distress syndrome (ARDS) which may extend to fatal acute lung injury—similar to the courses observed for the earlier coronavirus outbreaks caused by SARS-CoV-1 and by Middle East respiratory syndrome (MERS) coronavirus. Dampening the devastating overreaction of the immune system is a promising therapeutic intervention to limit tissue damage and prevent organ failure. Early attempts to broadly suppress the immune system using corticosteroids have yielded conflicting results that prompted the World Health Organization to initially advise against systemic administration. However, recent preliminary results from the RECOVERY trial (NCT04381936) showed that dexamethasone reduced 28-d mortality among patients receiving invasive mechanical ventilation or oxygen (Horby et al., 2020 *Preprint*) and gives a promising outlook on the benefit of immune-targeted therapeutics. With increasing knowledge of the immunopathogenic mechanisms of COVID-19, more selective measures targeting the inflammatory overreaction of the immune system may be taken without compromising humoral and cellular adaptive immunity, which are essential in clearing the virus and securing long-lasting immunity. While inhibition of cytokines as mediators of exacerbated inflammation is currently being investigated in clinical trials, we would like to highlight the potential use of CXCR2 antagonist to block migration of inflammatory myeloid cells, specifically neutrophils, into the infected lungs and other tissues.

Unbalanced immune response upon SARS-CoV-2 infection

The immune response upon infection exhibits characteristics that may explain the propensity toward a hyperinflammatory phenotype. SARS-CoV-1 and MERS-CoV were shown to circumvent immune recognition by several mechanisms including a potent inhibition of the IFN-inducing signaling pathways. Very recently, Blanco-Melo et al. (2020) demonstrated the same for SARS-CoV-2 using infection models in cell culture, in a ferret animal model, and in human COVID-19 serum samples. The inhibition of the IFN-mediated antiviral immune response, which would effectively block viral replication, leads to an unbalanced immune response with excessive secretion of pro-inflammatory cytokines and leukocyte attracting chemokines (Blanco-Melo et al., 2020), both of which may be targeted by therapeutic intervention.

Targeting pro-inflammatory cytokines

Elevated serum concentrations of pro-inflammatory cytokines and chemokines, especially IL-1β, IL-6, IL-8, TNF, G-CSF, and GM-CSF, have been described and were shown to correlate with disease severity in COVID-19 patients. The exacerbated cytokine release, also known as "cytokine storm," has drawn much attention and is being targeted in many clinical studies. Several clinical studies using the IL-6 receptor–blocking antibodies tocilizumab, sarilumab, or the IL-6–blocking antibody siltuximab are ongoing. IL-6 receptor inhibition is effectively used to relieve CAR-T cell therapy-induced cytokine storm, and the first anecdotal reports in COVID-19 treatment raise hope (Vabret et al., 2020). The list of therapeutic interventions targeting the overactive innate immune system is growing, with several studies investigating the IL-1 receptor antagonist anakinra or

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the JAK1/2 inhibitor ruxolitinib (Vabret et al., 2020).

Targeting tissue migration of inflammatory myeloid cells

The described mediators are increasingly produced as a result of massive infiltration of innate immune cells and their overactivation. Another approach may be to inhibit the migration of these proinflammatory immune cells to the site of infection in the first place to interrupt the self-propagating hyperinflammation and resulting tissue destruction. Inflammatory monocyte-derived macrophages are found amplified in the bronchoalveolar fluid of severe cases of COVID-19 (Liao et al., 2020) and may play an important role in the self-propagating inflammation by recruiting additional monocytes and neutrophils through high expression levels of the chemokines CCL2, CCL3, CCL5, CXCL8 (IL-8), CXCL9, CXCL10, and CXCL11 (Liao et al., 2020). First clinical studies using the CCR5 antagonist leronlimab (NCT04343651, NCT04347239) and maraviroc (NCT04441385, NCT04435522), originally developed against HIV infection, for the treatment of COVID-19 are ongoing or planned to block the migration of inflammatory monocytes to the site of infection. Besides monocytes, several findings hint toward an active involvement of neutrophils in disease pathogenesis. Neutrophils are known to play an important role in the establishment of lung injury and ARDS (Németh et al., 2020). The neutrophil effector functions—release of antimicrobial and inflammatory substances such as ROS, extracellular proteases like elastase and metalloproteinases, cationic peptides, and neutrophil extracellular traps (NETs)—act potently against extracellular pathogens like bacteria but may be less effective against intracellular pathogens like viruses. An errant overreaction may cause severe collateral damage in the lung: endothelial injury is followed by alveolar edema, NET-mediated inflammasome activation, platelet aggregation, and formation of microthrombi that are increasingly observed in COVID-19 patients (Bikdeli et al., 2020). The resulting diffuse alveolar damage with apparent macrophage and neutrophil infiltrates is indeed found in coronavirus-induced lung pathology (Barnes et al., 2020). Elevated concentration of markers of NET formation, such as cell-free DNA and myeloperoxidase-DNA, are detected in patients and sera from these patients are able to induce formation of NETs by neutrophils form healthy donors (Zuo et al., 2020 *Preprint*). In a recent Perspective, Barnes et al. (2020) suggest that aberrant NET formation may contribute to organ damage, thrombosis, and cytokine secretion and discuss NETs as a potential drug target. Further evidence for neutrophil involvement is given by the fact that neutrophil counts are increased in severe versus mild cases and the neutrophil-to-lymphocyte ratio is a predictive marker of mortality (Vabret et al., 2020).

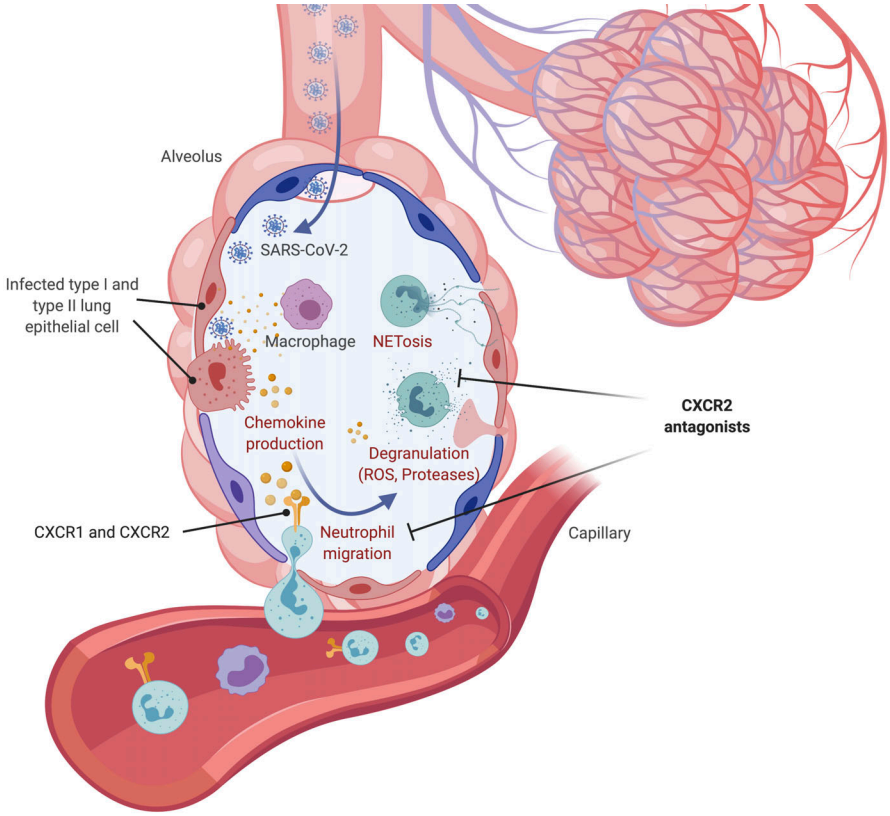

Figure 1. **Infiltration and activation of neutrophils into SARS-CoV-2–infected lungs.** Infected lung epithelial cells and inflammatory innate immune cells such as macrophages release the leukocyte attracting chemokines CXCL1, CXCL2, CXCL5, and CXCL8. Neutrophils are recruited and activated by these chemokines via their receptors CXCR1 and CXCR2. Subsequent release of antimicrobial effectors, such as ROS, proteases, and NETs, causes severe collateral damage in the lung. CXCR2 antagonists may inhibit lung damage by blockade of neutrophil migration and activation. Created with BioRender.

Neutrophils are mainly recruited via ligands of the CXC chemokine receptors CXCR1 and CXCR2. A plethora of ligands of these receptors have been described, namely CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8. CXCL8 (IL-8), a major neutrophil-attracting chemokine, is produced by lung epithelial cells upon infection and found to be highly expressed in patients with severe COVID-19 infection. Its serum levels correlate with disease severity (Vabret et al., 2020). One phase II study using the anti-CXCL8 (IL-8) drug BMS-986253 in cancer patients with COVID-19 was recently posted on ClinicalTrials.gov (NCT04347226). However, due to the high redundancy of ligands for CXCR1 and CXCR2, many of which are overexpressed in COVID-19 patients, blocking the receptors itself may be the more promising strategy (illustrated in Fig. 1).

Genetic deletion or therapeutic inhibition of CXCR2 has been reported to improve the course of many inflammatory lung disease models in mice. It was shown in an influenza-induced lung injury mouse model that a CXCR2 antagonist in combination with an antiviral treatment significantly improved clinical score and body weight change, decreased neutrophil count in BAL and reduced alveolar injury compared with antiviral treatment alone (Washburn et al., 2019). Importantly, another preclinical study found that CXCR2 deficiency prevents neutrophil accumulation in the lung in a

Table 1. **Clinical studies targeting recruitment of myeloid cells in patients with COVID-19**

Target	Identifier	Interventions	Status	Phase	Start date
CXCL8 (IL-8)	NCT04347226	BMS-986253	Recruiting	Phase II	April 2020
CCR5	NCT04343651	Leronlimab	Recruiting	Phase II	April 2020
	NCT04347239	Leronlimab	Recruiting	Phase II	April 2020
	NCT04441385	Maraviroc	Recruiting	Phase II	June 2020
	NCT04435522	Maraviroc	Not yet recruiting	Phase I	September 2020

https://www.clinicaltrials.gov; July 14, 2020.

murine influenza model without compromising viral clearance (Wareing et al., 2007). Several small-molecule antagonists blocking CXCR2 are under clinical investigation for the treatment of inflammatory diseases and cancer. The most developed ones, AZD5069 (AstraZeneca), navirixin (Merck Sharpe & Dohme), danixirin (GlaxoSmithKline), and the combined CXCR1/2 inhibitor reparixin (Dompé Farmaceutici S.p.A.) have completed phase I and II studies. Importantly, they displayed beneficial safety profiles without increasing risk of infection (Roberts et al., 2019; Lazaar et al., 2011; Hastrup et al., 2015; Miller et al., 2015). Studies with ozone- or LPS-induced airway inflammation in human volunteers showed that treatment with CXCR2 antagonists reduced neutrophil counts in the lungs by 50–80% (Lazaar et al., 2011; Leaker et al., 2013). For danirixin, it was shown in a phase IIa study in patients with acute, uncomplicated influenza that the treatment did not impede viral clearance (Roberts et al., 2019), again arguing that CXCR2 antagonism is a safe therapeutic option for virally induced lung injury. In addition to its effect on neutrophil migration, CXCR2 blockade also reduces activation of neutrophils and may therefore exert beneficial effects on a systemic level and reduce NET-induced thrombosis.

Thus, immunopathological evidence of neutrophil involvement in COVID-19, effectiveness of CXCR2 inhibition in preclinical models, and beneficial safety profile in patients with respiratory disease support the idea of applying CXCR2 antagonists in clinical trials for the treatment of severe COVID-19. CXCR2 ligand concentrations, e.g., CXCL1, CXCL2, CXCL5, and CXCL8, in sputum or bronchoalveolar lavage fluid of patients may be a useful biomarker for predicting an upcoming neutrophil infiltration with aggravation of symptoms in order to choose the ideal treatment start.

As of July 14, a search of the 2,553 studies for COVID-19 on https://www.clinicaltrials.gov disclosed very few studies targeting recruitment of myeloid cells (Table 1). None of the studies aim to inhibit neutrophil migration and activation via blockade of CXCR1 and CXCR2. This comment will hopefully spark discussions about using inhibitors of chemokine/chemokine receptor pathways to block excessive infiltration of neutrophils to interrupt the self-reinforcing hyperinflammation in severe cases of COVID-19 infection. Based on the current knowledge of immunopathology of COVID-19 and ARDS, there is strong evidence to investigate the usage of CXCR2 antagonists in the treatment of severe COVID-19.

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