

Interleukin-6, a major player of cytokine storm in COVID-19 and its alleviation by therapeutic antibodies

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Interleukin-6 (IL-6) is an important cytokine that plays a vital role in immune response and inflammation. Here, the signalling functions of IL-6 through its receptors, physiological and pathological roles, especially its contribution to various autoimmune diseases, cancers and severe COVID-19/SARS-CoV2 infections are described. It is reported that in severe COVID-19 infection and auto-immune diseases, the patients experience cytokine storms due to hyper-activation of the IL-6 receptor pathway leading to detrimental effects. Blocking IL-6 receptor action by therapeutic antibodies has been considered an attractive strategy of treatment. The latest findings on the application of anti-IL-6 therapeutic antibodies in COVID-19 patients are also discussed.

Keywords: COVID-19 infection, cytokine storm, immune response, interleukin-6, therapeutic antibodies.

Cytokines

CYTOKINES are low-molecular-weight (<30 kDa), non-immunoglobulin soluble glycoproteins acting non-enzymatically through specific receptors. They include lymphokines (lymphocyte-produced cytokines), monokines (monocyte-produced cytokines), chemokines (chemotactic-activated cytokines) and interleukins (IL, leukocyte-produced cytokines). Additionally, cytokines act and bring out cellular effects essentially in four different ways: include pleiotropy, redundancy, synergy and antagonism¹.

Based on their presumed function or target of action, cytokines are classified into six categories, viz. hematopoietins, IL, interferons (IFN), chemokines, tumour necrosis factor (TNF) and myokine.

SARS-CoV2 and the cytokine storm

The SARS-CoV2 virus caused the recent COVID-19 pandemic in December 2019. At the start of writing this article, it had affected countries all over the globe and about 530,896,347 cases were validated, of which 6,301,020

people had lost their lives². The major cause for this colossal loss is assumed to be the 'cytokine storm', also known as the cytokine storm syndrome (CSS)/cytokine release syndrome (CRS)³.

Several studies⁴ have shown that CRS is involved in COVID-19 infection. Additionally, there is an uncontrolled release of cytokines like IL-1, IL-6, IL-12 and IL-18, TNF, IFN and other inflammatory cytokines. This may increase alveolar–capillary gaseous exchange, lowering oxygen saturation in the pulmonary tissue (Figure 1)⁴.

Earlier, there were influenza-like symptoms after a systemic infection like sepsis and also due to immune therapies. This syndrome is currently known as cytokine storm. This terminology is used when immune dysregulation is characterized by the activation of macrophages and lymphocytes. This condition triggers an enormous amount of cytokine secretion, resulting in systemic inflammation and organ failure⁵.

Cells such as neutrophils, macrophages and natural killer (NK) cells are most often responsible for cytokine storm. In animal studies and for anticancer therapies in humans, the infusion of recombinant cytokines like IL-1, IL-6, IL-12, IL-18 and TNF- α causes severe effects or mortality aligned with the pivotal role of cytokines as mediators of hyper-inflammation. Several key cytokines which play a crucial role in the cytokine storm are IFN- γ , IL-1, IL-6, TNF- α and IL-18. The levels of IL-6 are highly elevated in various immunopathology disorders and cytokine storm⁵.

It has been estimated that the normal range of IL-6 in a healthy human is less than 7 pg/ml. Several patients who experience cytokine storm have shown IL-6 levels up to 100–10,000 pg/ml (ref. 6).

Interleukin-6

IL-6 is a cytokine that is not only a pro-inflammatory molecule, but also has anti-inflammatory properties. The formation of this cytokine is associated with several types of cells, including fibroblasts, keratinocytes, mesangial cells, vascular endothelial cells, mast cells, macrophages, dendritic cells, and T- and B-cells⁷.

The IL-6 receptor consists of two subtypes, namely gp80 and CD126. Two receptors are present in IL-6 – the

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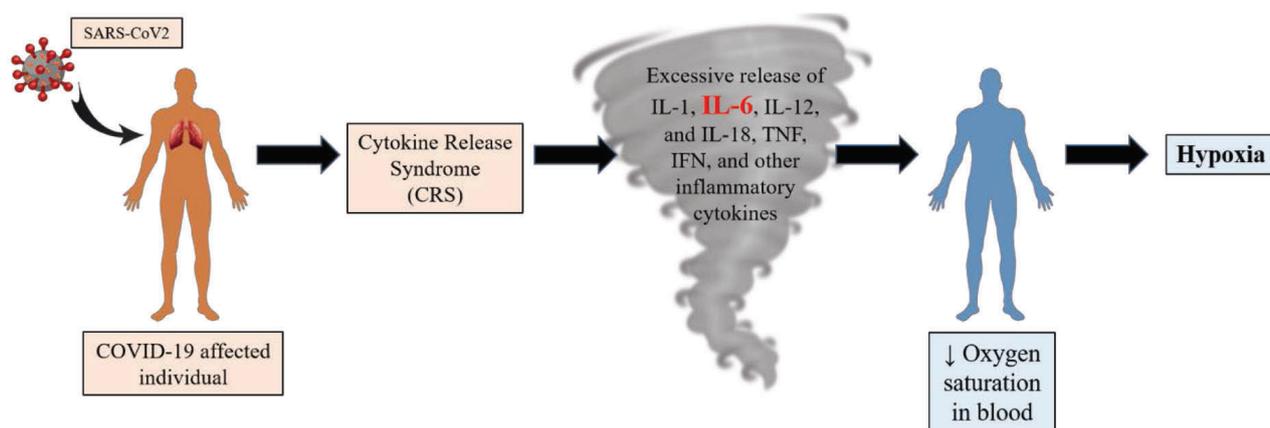


Figure 1. Effect of SARS-CoV2 on an individual leading to hypoxia. Here, the virus directly targets the angiotensin converting enzyme (ACE2) receptors in the lungs. This leads to the excessive release of cytokines, majorly IL-6, resulting in the cytokine release syndrome (CRS). Due to this, oxygen saturation level in the blood drops drastically causing hypoxia and ultimately leading to organ failure.

transmembrane IL-6 receptor (mIL-6R) located on the cell membrane and the circulating soluble IL-6 receptor (sIL-6R). These are formed by alternative splicing or cleaving of a disintegrin and a metalloproteinase 17/TNF- α converting enzyme (ADAM17/TACE) by metalloprotease⁸. mIL-6R is also IL-6Receptor-alpha (IL-6R- α), cell surface glycoprotein-80 (gp80), cluster of differentiation-126 (CD126) (ref. 9). IL-6 stimulates osteoclast formation when secreted by osteoblasts. Smooth muscle cells synthesize IL-6 as a pro-inflammatory reaction in the blood vessels. IL-6 also controls the development of proteins that are involved in the regulation of gene expression¹⁰. The pleiotropic character of IL-6 can be determined by the number of genes activated by its action. Hyper-production of IL-6 has been linked with numerous chronic diseases like rheumatoid arthritis (RA) and Castleman's disease. Thus, IL-6 and IL-6 receptors are the most analysed parameters for possible clinical cures for such diseases¹¹. They can also act as inflammatory markers in severe COVID-19 infection with poor prognosis.

According to recent data, the IL-6 family consists of seven members. They include IL-6, ciliary neurotrophic factor (CNTF), Leukaemia inhibitory factor (LIF), oncostatin M (OSM), cardiotrophin (CT-1), interleukin 11 (IL-11) and cardiotrophin-like cytokine (CLCF1)¹². All these members bind to their respective receptors and forms a four helix complex with gp130. Recently, IL-27, IL-35 and IL-39 have been included as new members of this group. These novel members are heterodimers and Epstein-Barr virus-induced gene 3 (*EBi3*) codes for their common protein subunit. *EBi3* belongs to the cytokine receptor family. Hence, the structure of these three new members is similar to IL-6/sIL-6R and IL-12 (ref. 13).

It has been noted that all the members have gp130 as the signal transducer and use the Janus kinase–signal transducer and activator of transcription (JAK–STAT) pathway for signal transduction as a quick response. STAT

are transcription factors which are activated by JAK that are present in the cytosol and then moved to the nucleus of the cell¹⁴.

Physiological and pathological roles of IL-6

A homodimer of gp130 is assembled when IL-6 binds with mIL-6R or sIL-6R. After binding, a hexamer network is established, comprising two units of IL-6, IL-6R and gp130. This complex further activates JAK, which phosphorylates tyrosine remnant in the cytoplasmic domain of gp130. This further phosphorylates STAT-3, which enters the nucleus and transmits the IL-6 signal¹⁵.

The whole process finally activates two signalling pathways (Figure 2).

- (i) The gp130 Tyr759-derived SHP-2/ERK MAPK pathway.
- (ii) The gp130 YXXQ-mediated JAK/STAT pathway.

Acute-phase response, angiogenesis, neutrophil trafficking, immune responses, bone metabolism, cartilage metabolism, anaemia of chronic diseases, cancer and lipid metabolism are some physiological and pathological roles of IL-6 (ref. 16).

Acute-phase response

During this process, the pathogens are neutralized and their further invasion is restricted, thereby minimizing tissue damage. As noticed in the inflammatory conditions of humans, a wide spectrum of acute-phase proteins such as synthesis and secretion of C-reactive protein (CRP), fibrinogen, serum amyloid A (SAA), α -1-antitrypsin, α -1-antichymotrypsin and haptoglobin show enhanced levels. Interestingly, fibronectin, albumin and transferrin are

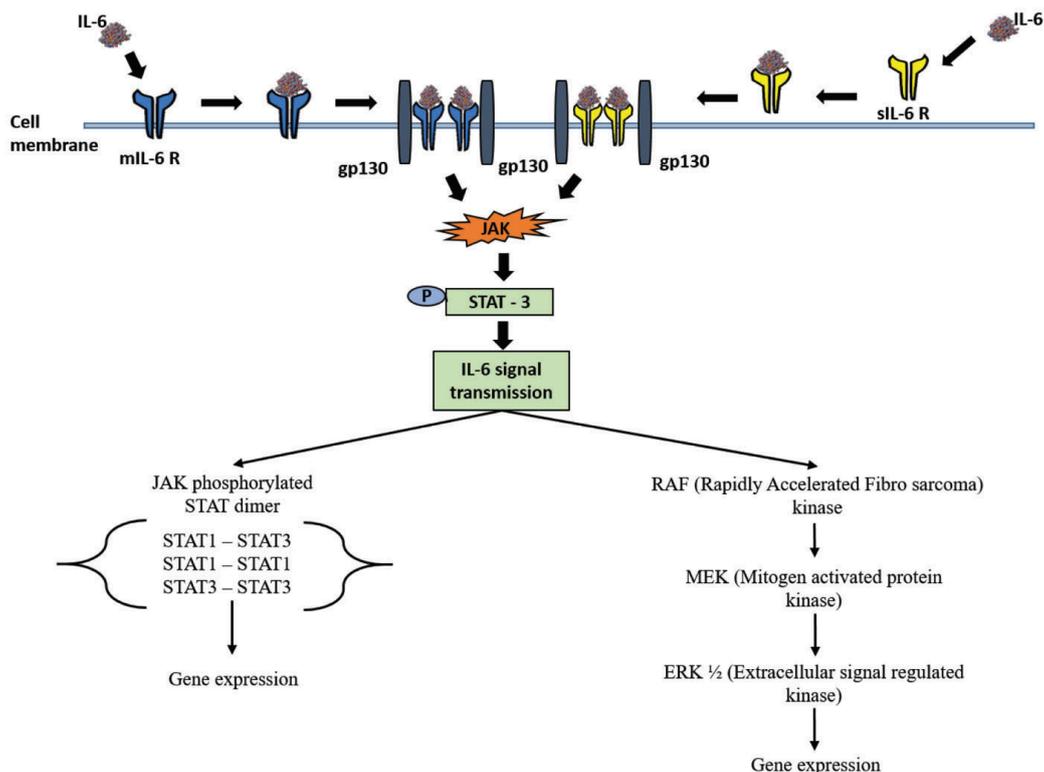


Figure 2. Mechanism of action of IL-6 when attached with mIL-6R and sIL-6R. The blue receptor is attached to the membrane and when IL-6 binds to it. This is called classic *cis* signalling. The yellow receptor is a soluble receptor and when IL-6 attaches to this, it is called *trans* signalling. Signal transmission takes place through the two pathways shown.

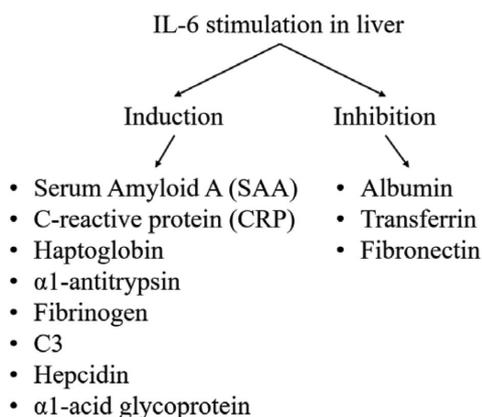


Figure 3. Acute phase response of IL-6.

diminished, as shown in Figure 3, while the entire process is promoted by IL-6 (ref. 17). Injection of IL-6 may lead to various effects on an individual depending upon the type of injection. For example, an intracerebroventricular injection may increase the body temperature, whereas an intravenous or intraperitoneal injection will not affect body temperature.

SAA is an acute-phase protein which is produced by IL-6 stimulation in the liver. In the event of inflammation, SAA replaces apo-lipoprotein and circulates with high-

density lipoprotein (HDL). Once the process is completed, SAA detaches from HDL and undergoes degradation. Due to this, SAA gets deposited in the extracellular region of critical tissues as amino acid fibrils, which results in renal breakdown and gastrointestinal tract malfunction. This cytokine plays an important role in the synthesis of hepcidin in the liver and is involved in regulating iron recycling in the spleen and absorption in the intestine, leading to iron deficiency anaemia¹⁵.

Angiogenesis

The production of blood vessels is known as angiogenesis, which is an essential component of inflammation. IL-6 has been indicated to play a part in pathogenic angiogenesis in disorders like stroke, RA and various cancers. It has been noted that vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), transforming growth factor- β (TGF- β), IL-6, IL-8, IL-1 and TNF- α show angiogenic activity. Also, a significant increase in VEGF may lead to RA pathogenesis¹⁸. Elevated concentrations of IL-6 and CRP have been linked with age-related macular degeneration (AMD). Blocking of IL-6 also suppresses choroidal neovascularization (CNV), the formation of new blood vessels originating from the choroid, resulting in vision loss.

Neutrophil trafficking

IL-6, which signals through the typical gp130 receptor subunit, is a key control point for neutrophil trafficking along the inflammatory response¹⁹. Also, IL-6 acts as a stimulus for myelopoiesis, i.e. formation of tissues in the bone marrow. Neutrophils drift towards the inflamed location leading to the robust synthesis of inflammatory moderators like prostaglandins, complements, reactive oxygen species (ROS), proteases and cytokines. Additionally, IL-6 augments the expression of adhesive molecules like vascular cell adhesion molecule (VCAM1) and intercellular adhesion molecule (ICAM1). This, in turn, induces chemokines such as CXCL-8 (CX chemokine ligand)/IL-8, CCL2/MCP-1 (monocyte chemoattractant protein), CCL3/MCP-3 and CCL20, leading to a reduction in moving neutrophils in RA.

Immune response

IL-6, which is produced rapidly and temporarily in response to infections and tissue injury, assists host defence by activating acute process responses, haematopoiesis and immunological reactions²⁰. As known, IL-6 plays a crucial role in B-cell differentiation. This complements the synthesis of IgM, IgG and IgA in B-cells triggered with *Staphylococcus aureus* Cowan I or pokeweed mitogen. IL-6 also encourages antibody synthesis by developing the B-cell helper competence of CD⁴⁺ T-cells, i.e. enhances humoral immunity. IL-6 also causes B-cell irregularities linked with the inflammatory procedure. IL-6 trans-signalling was also found to be responsible for the induction of pre B-cell enhancing factor (PBEF) in synovial fibroblast cells. Blockage of IL-6 suppresses the anti-CD28 antibody activated CD⁴⁺ T-cell proliferation. This is due to the introduction of regulatory T-cells (T_{reg}) and suppression of IL-2 signalling. With regard to cytokine production, CD⁴⁺ helper T-cells can be divided into T_{h1} and T_{h2} . IL-6 increases T_{h2} development caused by IL-4, while inhibiting T_{h1} differentiation induced by IL-12.

Bone metabolism

In RA and other bone disorders, IL-6 plays a key role in aberrant bone resorption. Synovial fluid from RA patients has elevated levels of IL-6, sIL-6R and the IL-6-sIL-6R network that trigger the development of osteoclasts. IL-6 suppresses the development of osteoclast precursor cells by regulating transcription linked with MAPK phosphatases and the ubiquitin pathway. This IL-6 induced suppression can be reversed by adding sICAM-I to a monoculture system. At the cellular plane, bone estrogen reduces osteoclastic differentiation, thereby decreasing the number of osteoclasts and the functioning remodelling factors. This influence is presumably moderated by certain cytokines,

with the most potent candidates being IL-1 and IL-6, through a yet unexplained mechanism. The presence of IL-6 in bone marrow cells is controlled by estrogen²¹.

It has been observed that a regular number of osteoclasts and normal active bone reabsorption were present in IL-6-deficient mice. IL-6 is not required for normal bone growth and osteoclastogenesis or osteoclastic bone-resorbing activity. The relevance of IL-6 in post-menopausal osteoporosis is debatable because bone deterioration has been shown in individuals with estrogen shortage post-menopausal osteoporosis, and the anti-IL-6 antibody remedy is ineffective in prohibiting bone destruction²².

Cartilage metabolism

An increase in IL-6 and a decrease in insulin-like growth factor (IGF-1) lead to growth impairment, including juvenile RA, Crohn's disease, cystic fibrosis and immunodeficiency. The endochondral ossification induces lengthwise bone growth in the growth plate, which is monitored by aggregation of generic and hormonal dynamics, growth factors, the surroundings and diet. Studies have shown that IL-6 suppresses insulin-induced differentiation of chondrogenic pioneer cells. These cartilage cells are eventually transformed into benign tumours²³. This can be inhibited by a reduction in the levels of IGF-1. IL-6 encourages the synthesis of matrix metalloproteinases (MMPs) such as MMP1, MMP3 and MMP13, and disintegrin and metalloproteinase with thrombospondin motif-4 (ADAMTS) from chondrocytes and synovial cells. In RA, IL-6 has been linked to cartilage degradation that was found to be based on IL-6 in murine models of RA²⁴.

Contribution of IL-6 in autoimmune diseases and the role of therapeutic antibodies

Autoimmune diseases include a group of illnesses that are poorly understood, as both genetic and environmental factors are implicated in the pathogenesis. Autoimmune disorders are classified into tissue-specific and systemic disorders²⁰. Cytokines, such as ILs and IFNs, are produced by a variety of immune cells, which are involved in the regulation of physiological and pathophysiological processes. Studies have demonstrated the critical roles of pro-inflammatory cytokines, including TNF, IL-1 and IL-6, in the development of RA²⁵. In certain cases, IL-6 has been persistent in those with immune-inflammatory illnesses, including Castleman's disease and RA. Administration of anti-IL-6 receptor antibodies is effective in a few patients with RA. This corroborates the findings in animal models, which suggested anti-IL6R antibody treatment as effective in IL-6-related immune diseases.

The complementarity determining regions (CDRs) of the mouse anti-human IL-6R antibody were transplanted onto human IgG1 to develop tocilizumab, an IgG1-class,

humanized anti-IL-6R monoclonal antibody. By suppressing IL-6 attachment to both mIL-6R and sIL-6R, this antibody inhibited IL-6-mediated signal transduction²⁶.

Tocilizumab in rheumatoid arthritis

It has been observed in clinical studies that tocilizumab successfully suppresses disease activity and defends against the worsening of joint damage, and increases everyday activity and the quality of life²⁶. While other biologics, including TNF inhibitors, a T-cell stimulus inhibitor and a B-cell diminishing mediator, are also used to treat RA patients, tocilizumab is the most potent in controlling disease activity. Tocilizumab monotherapy seems to be the only biologic known to be more promising than the conventional drug methotrexate (MTX)²⁷.

Tocilizumab in case of systemic juvenile idiopathic arthritis

Systemic juvenile idiopathic arthritis (sJIA) is a long-lasting infant arthritis subset that contributes to systemic inflammation, joint damage, physical weakness and development deficiency. IL-6 in the blood of patients had increased significantly and it was well-linked with disease activity²⁷. A total of 112 children with dynamic SJIA were arbitrarily injected with placebo or tocilizumab in a worldwide stage-III clinical trial. After treatment, tocilizumab showed promising results at the beginning of a novel phase in curing untreated juvenile diseases²⁸.

IL-6 in systemic lupus erythematosus

IL-6 may play a direct role in tissue injury, according to several studies²⁹. Systemic lupus erythematosus (SLE) patients had higher blood IL-6 levels in some, but not all studies. Patients also showed a greater frequency of IL-6 secreting peripheral blood mononuclear cells (PBMCs) than healthy controls. Lymphoblastoid cells collected from SLE patients generated large amounts of IL-6 and inhibiting IL-6 *in vitro* decreased the anti-dsDNA autoantibody synthesis²⁹. Similar observations were made when autoreactive T-cells and autologous B-cells were given an anti-IL-6R monoclonal antibody.

IL-6 in multiple sclerosis

Multiple sclerosis (MS) has been linked to increased levels of IL-6 in the bloodstream. In the plasma of individuals with acute meningoencephalitis, elevated levels of IL-6 can be seen. In the majority (91%) of MS patients, IL-6 is detectable in their plasma with a mean value of 54 U/ml (ref. 30). The interplay of T- and B-cells has been suggested in the formation of MS. In the early stages of MS,

Th17 cells are considered to have a significant impact³¹. Elevated differentiation of plasma cells of activated B-cell replicas is implicated in the formation of tissue-destructing autoantibodies.

IL-6 in cancer

IL-6, one of the key cytokines in the tumour microenvironment, is a crucial component present in elevated levels and is considered to be unregulated in cancer. In almost all forms of tumours, its overexpression has been observed. Chronic inflammation in the tumour microenvironment has been found to promote tumour development and cause resistance to chemotherapy and radiotherapy. Numerous cancers, like breast cancer, human prostate cancer, ovarian cancer and lung cancer, have been found to overexpress IL-6 in the tumour microenvironment³². A major part is contributed by IL-6 in the maintenance and development of tumour cells³³. It is also classified as a multipurpose cytokine, the chief signalling pathway stimulator of JAK-STAT3. STAT3 is used as an oncogene in many cancers and has been shown in several models to induce malignant cell transformation. This tends to escalate in prostate cancers³⁴. IL-6 has been shown to specifically induce increased cellular invasions over basement membrane degeneration induced by the gene expression of matrix metalloproteinase. This indicates a vital role of IL-6 in the occurrence of various cancers. In particular, IL-6 has been proposed as a potential therapeutic target for managing and curing these cancers.

Role of IL-6 in COVID-19 patients

IL-6 has gained prominent recognition in recent years as a result of the emergence of the corona-virus and its impact on the pathogenesis of COVID-19. In patients with this disease, cytokine storm is one of the most important pathological conditions³⁵. The pathogenesis of COVID-19 disease remains uncertain and data for potential therapeutics have just started emerging. The COVID-19 pathogenesis for severe acute respiratory syndrome includes TNF- α , IFN- γ , IL-1 and IL-12 serum cytokines and chemokine IL-6 (ref. 36). The IL-6 is one of the main cytokines contributing to an inflammatory storm, which may result in an increased instability of the alveolar capillary and blood-gas exchange. In this inflammatory process, IL-6 tends to be the dominant cytokine³⁷. Flushing of virus from the body, its perseverance and spread, and prolonged viral infection are some major impacts due to the imbalance of IL-6 (ref. 38). High levels of pro-inflammatory cytokines such as IL-1, IL-6, IL-18 and TNF- α may be seen in severe cases. When a patient experiences a cytokine storm, CRP is produced at high levels (>100 mg/l) by hepatocytes in response to IL-6. The peak serum level of ferritin reached more than 1000 μ g/l, an indicator of macrophage

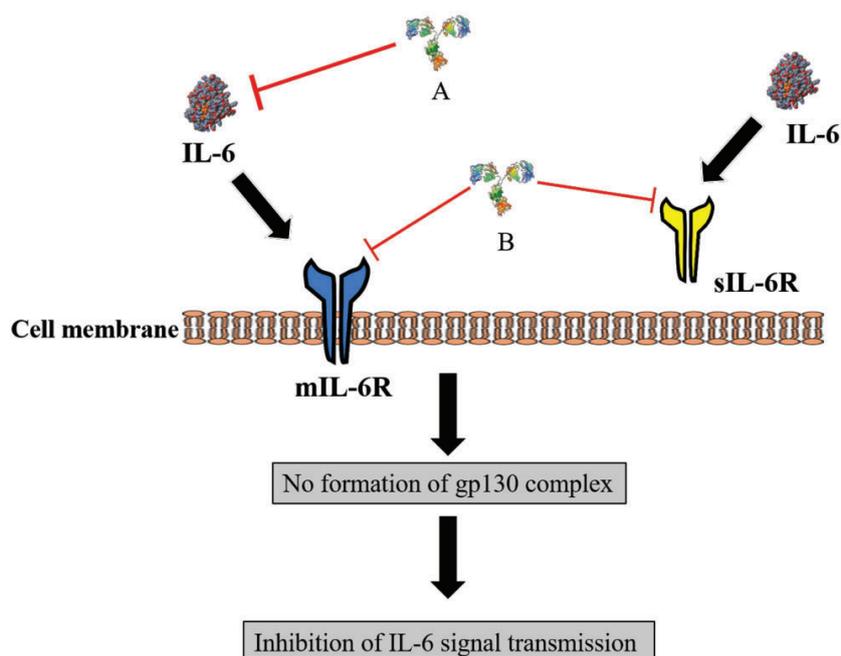


Figure 4. Mechanism showing the working of anti-IL-6 and anti-IL-6 receptor drugs. 'A' denotes anti-IL-6 drugs which bind to IL-6. 'B' signifies anti-IL-6 receptor drugs which bind to the IL-6 receptor and block the binding of IL-6 molecule to the receptor. Both the drugs ultimately halt the formation of the gp130 complex, resulting in the inhibition of IL-6 signalling.

activeness. A German study has shown that IL-6 levels >80 pg/ml together with CRP levels >97 mg/l can lead to respiratory failure and death⁶.

IL-6 in COVID-19 pathogenesis

In viral infections, the conflicting functions of IL-6 may be challenging, mainly in choosing potential therapeutic options. Laboratory experiments have shown that anti-viral immune reactions are inefficient and the contagion weakens after a collapse in cytokine levels, specifically IL-6. These studies also reveal that IL-6 might be essential for virus removal through the modulation of lymphocyte responses, quenching inflammation, inducing the repair of lung tissue, restricting macrophage invasion and phagocytosis³⁹.

Lymphopenia and cytokine storms have been identified as two significant immunopathological consequences of coronavirus infection (i.e. SARS-CoV, MERS-CoV and SARS-CoV-2)³⁴. The amount of serum IL-6 was substantially elevated after disease progression, which can be linked with a wide variety of pulmonary abrasions that have been reflected in the computed tomography (CT) scans.

The literature survey carried out for this review has clearly indicated that IL-6 and its receptors are the major cytokines that are elevated in almost all COVID-19 patients. So, it can be a promising therapeutic target for dealing with this viral infection.

Treatments involving blockage of IL-6 and IL-6 receptors

The therapeutic potential for COVID-19 signal inhibitors is unfolding rapidly because the disease exhibits signs of both pro-inflammatory and autoimmune disorders⁴⁰. IL-6 has been shown as the key driver of cytokine storm in severely affected individuals with COVID-19 infection. To overcome this, it is critical that selective blocking of the cytokine is further explored with drugs such as IL-6 inhibitors⁴¹. This may provide therapeutic solutions to the cytokine storm and reduce the damages caused by inflammation⁴². IL-6 receptor blockers are officially sanctioned for a variety of autoimmune disorders and are generally known to be well-tolerated and safe. Thus, they are also tested for alleviating the damages caused due to COVID-19 infection⁴³.

Figure 4 shows the mechanism of action of these anti-IL-6 and anti-IL-6 receptor drugs. The blocking of IL-6 and its receptors leads to the inhibition of IL-6 signalling. Table 1 shows some IL-6 and IL-6R blockers specifically targeted towards the remediation of COVID-19 infection.

Tocilizumab: A Japanese pharmaceutical company, Chugai Pharmaceutical Ltd, developed this drug – an anti-IL-6R monoclonal antibody – as a substitute therapy for individuals suffering from COVID-19 exposed to cytokine floods¹⁵. This is a humanized monoclonal antibody which works in contradiction to the human IL-6 receptors and restricts the

Table 1. Few interleukin-6 (IL-6) and IL-6R inhibitors targeting COVID-19 infection

| Monoclonal antibody and brand name (in brackets) | Target | Nature of antibody | Use | Current status | Clinical trial number and sponsor company | Reference |
|--|--------|--------------------|--------------------|----------------|--|-----------|
| Tocilizumab (Actemra [®]) | IL-6R | Humanized IgG1 | COVID-19 pneumonia | Phase 3 | NCT04356937, Massachusetts General Hospital, USA | 45 |
| Levilimab (Ilsira [®]) | IL-6R | Human IgG1 | Severe COVID-19 | Phase 3 | NCT04397562, Biocad, Russia | 47 |
| Olokizumab (Artlegia [®]) | IL-6 | Humanized IgG4 | Severe COVID-19 | Phase 2/3 | NCT04380519, R-Pharm International, LLC, France | 56 |
| Sarilumab (Kevzara [®]) | IL-6R | Human IgG1 | COVID-19 | Approved | NCT04327388, Sanofi | 57 |
| Sirukumab (Plivensia [®]) | IL-6 | Human IgG1 | COVID-19 | Phase 2 | NCT04380961, Janssen Pharmaceutica N.V., Belgium | 54 |

biological action of IL-6R by constraining its attachment to the receptor.

Observations have confirmed the efficacy of tocilizumab in preventing COVID-19-induced cytokine storms. Acute-phase reactant levels had declined in several patients and they had entered a steady state leading to a decline in IL-6 levels. Several traditional agents such as corticosteroids are also used to treat cytokine storms with limited success⁴⁴.

In a clinical trial (NCT04356937), the prescribed amount of tocilizumab (4–8 mg/kg) delivered every four weeks as a single intravenous infusion benefits the patients. In several other trials, tocilizumab was shown to have a beneficial impact in combination with antiviral drugs such as lopinavir/ritonavir (400 mg/100 mg twice daily) or remdesivir 100 mg/daily and corticoids that can boost the patients' clinical outcomes. The timing of the prescription of tocilizumab and the identification of eligible patients for treatment were the important concerns. Patients with serious disease progression were involved in all of the trials analysed. Some researchers mention that for best results, tocilizumab must be administered at the start of inflammation and the first stages of decline in O₂ saturation. However, the exact timing and phase of infection for initiating this treatment is still unknown^{45,46}.

Levilimab: This is an immunomodulatory drug with the brand name Ilsira[®], now under study with clinical trial number NCT04397562. The manufacturer, Biocad, Russia claims this drug to be helpful in the treatment of patients with severe COVID-19 infection and some autoimmune disorders by blocking IL-6R (ref. 47). This drug was initially manufactured to treat RA, but the latest reports reveal that it has been approved for the treatment of COVID-19 in Russia. It acts as an antagonist for IL-6R by neutralizing the cytokine storm^{48,49}.

Olokizumab: This immunomodulatory drug acts against IL-6 in RA and has been recently used in individuals with serious COVID-19 infection. The clinical testing of this drug is in phase-III with trial number NCT04380519. It has been shown that this drug tends to nullify both *cis* and *trans* signalling of IL-6 in the cells. Nullification of *cis*-signalling was done with CRPs and SAA established in

primary human hepatocytes, where membrane gp80 was expressed. The neutralization of *trans* signalling was done in human umbilical vein endothelial cells, where the soluble form of gp80 was essential for the phosphorylation of STAT^{50,51}.

Sarilumab: This is a fully human monoclonal antibody which acts in contrast to IL-6R. This antibody was primarily developed for the treatment of RA and now it is being tested in COVID-19 patients. The clinical testing number is NCT04327388. Sarilumab works by blocking the *cis* and *trans* signalling of IL-6, thus controlling the cytokine storm. It was manufactured by Sanofi and Regeneron Pharmaceuticals, France and was approved by US FDA in 2017 (ref. 51). The drug was launched in the market under the brand name Kevzara[®]. It can be used in combination with methotrexate or individually in case a patient is sensitive to the latter^{52,53}.

Sirukumab: This is a monoclonal antibody against IL-6, developed by Janssen Pharmaceutica N.V., Belgium. It binds to human IL-6 with high affinity, inhibiting IL-6-mediated signalling as well as the biological effects of IL-6. The phase-II clinical trial (NCT04380961) of this monoclonal antibody was completed on 24 June 2021, which included 212 randomly selected participants⁵⁴.

Conclusion and future directions

With the emergence of new mutations frequently in SARS-CoV2, the COVID-19 infection may continue in the coming years. Hence there is a paramount need for proper treatment of this disease. Vaccines play a significant role in fighting against the virus. However, the new strains with novel mutations can escape and invade the human immune system. As discussed in this study, IL-6 play the foremost part in a cytokine storm, which is a major issue in COVID-19 infection. Antibody therapies may also have potential applications not only against this virus, but also for other deadly viruses inducing cytokine storm.

Recent studies have indicated that anti-IL-6R inhibitor tocilizumab is beneficial in preventing the progression of COVID-19 disease⁵⁵. However, it did not improve survival

in a single treatment, so additional clinical interventions are required. Several studies have focused on using tocilizumab. The effectiveness of the treatments could be evaluated with several other IL-6R or IL-6 inhibitors listed in Table 1.

Most of these inhibitors are full antibodies and there is a need to evaluate safety of patients. Moreover, the cost of production of these antibodies is high. In addition, the demand for these inhibitors could be high given that the COVID-19 pandemic still exists and has been predicted to affect humans for a few more years. Since the treatment is expensive, there is an urgent need for developing antibody fragments such as Fab or single-chain antibody fragments of anti-IL-6 or anti-IL-6R, which can easily be produced in prokaryotic expression systems such as *Escherichia coli*. In conclusion, IL-6 and IL-6R inhibitors hold promise in treating COVID-19 patients in combination with other clinical treatment options.

Conflict of interest: The authors declare that there is no conflict of interest.

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