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Associations between immune-suppressive and stimulating drugs and novel Covid-19 – a systematic review of current evidence

Beth Russell^{a*}, Charlotte Moss^{a*}, Gincy George^{a*}, Aida Santaolalla^{a*}, Andrew Cope^{b,c}, Sophie Papa^{b,d**}, Mieke Van Hemelrijck^{a**}

^a King's College London, Translational Oncology and Urology Research, London, UK

^b Guy's and St. Thomas NHS Foundation Trust, London, UK

^c King's College London, Centre for Rheumatic Diseases, London, UK

^d King's College London, School of Cancer and Pharmaceutical Sciences, London, UK

*All authors contributed equally

**Both senior authors contributed equally

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Abstract

Background: Cancer and transplant patients with Covid-19 have a higher risk of developing severe and even fatal respiratory diseases, especially as they may be treated with immune-suppressive or immune-stimulating drugs. This review focuses on the effects of these drugs on host immunity against COVID-19.

Methods: Using Ovid MEDLINE, we reviewed current evidence for immune suppressing or stimulating drugs: cytotoxic chemotherapy, low dose steroids, TNF-alpha blockers, IL-6 blockade, JAK inhibitors, IL-1 blockade, mycophenolate, tacrolimus, anti-CD20 and CTLA4-Ig.

Results: 89 studies were included. Cytotoxic chemotherapy has shown to be a specific inhibitors for SARS coronavirus in in vitro studies, but no specific studies exist as of yet for COVID-19. No conclusive evidence for or against the use of NSAIDs in the treatment of COVID-19 patients is available, nor is there evidence indicating that TNF α blockade is harmful to patients in the context of COVID-19. COVID-19 has been observed to induce a pro-inflammatory cytokine generation and secretion of cytokines such as IL-6, but there is no evidence of the beneficial impact of IL-6 inhibitors on the modulation of COVID-19. Although there are potential targets in the JAK-STAT pathway that can be manipulated in treatment for coronaviruses and it is evident that IL-1 is elevated in patients with a coronavirus, there is currently no evidence for a role of these drugs in treatment of COVID-19.

Conclusion: The COVID-19 pandemic has led to challenging decision making about treatment of patients critically unwell. Low dose prednisolone and tacrolimus may have beneficial impact on COVID-19. The mycophenolate mofetil picture is less clear, with conflicting data from pre-clinical studies. There is no definitive evidence that specific cytotoxic drugs, low dose methotrexate for auto-immune disease, NSAIDs, JAK kinase inhibitors or anti-TNF α agents are contraindicated. There is clear evidence that IL-6 peak levels are associated with severity of pulmonary complications.

Introduction

Since the outbreak of SARS-CoV-2 or COVID-19 coronavirus started in China in December 2019, there is increasing evidence that those with existing comorbidities, older age or a compromised immune system are at higher risk of developing severe and even fatal respiratory diseases (1). Cancer and transplant patients are also considered to be in this risk group (2, 3), especially as they may be treated with immune-suppressive or immune-stimulating drugs.

The current review focuses on the effects of immune-suppressive or immune-stimulating drugs on the host immunity against COVID-19. Here we report a short introduction to each drug, followed by a summary of the results from the identified studies for each relative drug.

We hypothesise that the drugs selected will likely to be categorised into one of two groups: 1) those that may be harmful for patients and put them at risk of increased morbidity/mortality associated with COVID-19 infection and 2) those that may be used to treat the immunopathology associated with severe persistent viral infection. The clinical impact of this review is thus two-fold. It aims to identify which drugs clinicians should be thinking about taking patients off to protect them from increased harm from COVID-19 and also which drugs could be potentially beneficial in the fight against the disease.

This review covers the information available today. As the COVID-19 pandemic progresses there is an opportunity and responsibility to collect prospective data using established randomised controlled trial involving drugs of interest and cohort based translational studies.

- 1) All cytotoxic chemotherapy
- 2) Low dose steroids and NSAIDs
- 3) Any TNF blocker
- 4) IL-6 blockade
- 5) JAK inhibitors
- 6) IL-1 blockade
- 7) Mycophenolate
- 8) Tacrolimus
- 9) Anti-CD20
- 10) CTLA4-Ig

Methods

A search was conducted using Ovid MEDLINE and a total of 89 studies were included in this review for 10 different types of immune suppressing or immune stimulating drug groups. Table 1 shows the search terms and the number of studies included in the review.

Table 1. Search terms and the number of studies included for each investigated drug group.

Drug group	Search terms	Number of studies included
All cytotoxic chemotherapy	(SARS-CoV-2 or coronavirus or COVID or betacoronavirus or MERS-CoV or SARS-CoV) AND (chemotherapy)	24/30
Low dose steroids/NSAIDs	(SARS-CoV-2 or coronavirus or COVID or betacoronavirus or MERS-CoV or SARS-CoV) AND (anti-inflammatory or ibuprofen or isobutylphenylpropionic acid or cortisone or non-steroidal anti-inflammatory)	13/58
Any TNF blocker	((SARS-CoV-2 or coronavirus or COVID or betacoronavirus or MERS-CoV or SARS-CoV) AND (TNF blocker or Anti-TNF therapy or TNF-alfa inhibitor or infliximab or etanercept or Certolizumab or Golimumab or adalimumab))	2/3

IL-6 blockade	Two different search strategies were explored due to the number of agents that block the IL-6: 1. ((SARS-CoV-2 or coronavirus or COVID or betacoronavirus or MERS-CoV or SARS-CoV) AND (Anti-IL-6 therapy or anti-interleukin-6 receptor antibody or anti-interlukin-6 therapy or interleukin 6 blockage or interlukin-6 blockage or IL 6 blockage or IL-6 blockage or tocilizumab or siltuximab or Sylvant or sarilumab or olokizumab or CDP6038 or elsilimomab or BMS-945429 or ALD518 or sirukumab or CNTO 136 or CPSI-2364 or ALX-0061 or clazakizumab or olokizumab or sarilumab or sirukumab or ARGX-109 or FE301 or FM101)) 2. ((SARS-CoV-2 or coronavirus or COVID or betacoronavirus or MERS-CoV or SARS-CoV) and (IL-6 or interleukin 6 or Anti-IL-6 therapy or IL-6 blockage or tocilizumab or siltuximab))	1. 0 2. 23/108
JAK inhibitors	((SARS-CoV-2 or coronavirus or COVID or betacoronavirus or MERS-CoV or SARS-CoV) and (JAK or JAK1 or JAK2 or TYK3 or tofacitinib or baricitinib or filgotinib or peficitinib or ABT494 or decernotinib))	4/15
IL-1 blockade	(SARS-CoV-2 or coronavirus or COVID or betacoronavirus or MERS-CoV or SARS-CoV) AND (Interleukin-1 or IL-1 or IL-1RA or canakinumab or anti-IL-1 or IL-1 antagonists or IL-1 blockers or rilonacept or IL-1 trap or ACZ885 or anakinra)	9/37
Mycophenylate	(SARS-CoV-2 or coronavirus or COVID or betacoronavirus or MERS-CoV or SARS-CoV) AND (mycophenolate mofetil OR mycophenolate OR myfortic)	13/29
Tacrolimus	(SARS-CoV-2 or coronavirus or COVID or betacoronavirus or MERS-CoV or SARS-CoV) AND (envarsus or tacni or tacrolimus or prograf or FK506)	3/18
Anti-CD20	((SARS-CoV-2 or coronavirus or COVID or betacoronavirus or MERS-CoV or SARS-CoV) AND (anti-cd20 monoclonal antibodies or anti-cd20 or rituximab or truxima or zevalin or ruxience or rituxan or arzerra or gazyva)	0
CTLA4-Ig	((SARS-CoV-2 or coronavirus or COVID or betacoronavirus or MERS-CoV or SARS-CoV) AND (CTLA-4 Ig or CTLA4 or CTLA-4 or Ipilimumab or yervoy)	0

Results

1. All cytotoxic chemotherapy

Cytotoxic chemotherapy inhibits cell division through multiple mechanisms. It may have therapeutic activity as compounds against corona viral strains. Moreover, cancer patients who have undergone chemotherapy may be at increased risk of developing symptoms of severe acute respiratory syndrome.

Results

The search yielded 24 results, of which 18 were included (Supplementary Table 1). Below are key case-reports of interest to highlight:

- Coronavirus was one of the most common viral pathogens identified in paediatric cancer patients undergoing chemotherapy, second to human rhinovirus (4). Viral co-detection was frequent in patients with cancer and acute respiratory infections.
- A brain biopsy was HCoV-OC43-positive by metagenomic next-generation sequencing in the case of a one-year-old child with pre-B acute lymphoblastic leukaemia (5).
- A case of a young woman with stage IIIA breast cancer from 1999 reported that diagnosis of coronavirus following treatment for cancer using a high-dose chemotherapy regimen and autologous bone marrow and stem cell transplantation. The electron microscopy revealed coronavirus pneumonia (6). Coronavirus should be potentially considered in the differential diagnoses of respiratory failure in patients who have undergone high dose chemotherapy and autologous bone marrow transplantation.

In addition, some further observations have been made in relation to specific treatments:

- Although HIV protease inhibitors such as lopinavir has been suggested as a low micromolar inhibitor of MERS-CoV, the different mechanistic classes that HIV and

coronavirus fall under meant that there was low affinity for coronavirus strains compared to HIV (7).

- The following compounds have been shown to be active in vitro against the SARS-CoV virus: TNF- α -converting enzyme inhibitor (TAPI-2); IFN- α (B/D, mDEF201 by adenovirus 5 vector, CR3014 humanized monoclonal antibody (a neutralising antibody specific for SARS-CoV), recombinant IFN- α 2b and type I IFN- β); Interferon inducers (Ampligen and polyinosinic–polycytidylic); therapeutic antibodies (2978/10, equine anti-SARS-CoV F[ab'] and monoclonal antibody 201); attachment inhibitors (Urtica Dioica lectin and griffithsin); host immune system (8).
- 6-mercaptopurine (6MP) and 6-thioguanine (6TG) have been used in cancer chemotherapy for treatment of acute lymphoblastic or myeloblastic leukaemia and were found to be specific inhibitors for the SARS coronavirus (9).
- Carbohydrate-binding agents (CBA) may be able to block enveloped viruses other than HIV in their entry process and coronaviruses and influenza viruses are other examples of enveloped viruses that may be highly susceptible to the antiviral action of CBAs (10).
- The genome of SARS-CoV encodes five major proteins: the spike protein (S), the envelope protein (E), the membrane glycoprotein (M), and the nucleocapsid protein (N). M and E may help host cells to induce the production of protective IFN- α to fight against the virus. Bananin 1-[3-hydroxy-5-(hydroxymethyl)-2-methyl-4-pyridinyl]-2,8,9-trioxadadamantane-3,5,7-triol acts as zinc (Zn²⁺) chelator and is therefore of interest to target and inhibit immunodeficiency virus type 1 (HIV-1) zinc finger HIV-1 RNA-binding nucleocapsid protein p7 (NCp7). Bananin is converted to bananin 5'-monophosphate (BNP) which together with B6RA (vitamin A-vitamin B6 conjugate) and could inhibit infectious virion encapsidation. Targets of BNP and B6RA has shown to be present also in SARS-associated coronavirus making them possible therapeutic candidates (11).

Conclusion

Coronavirus strains were one of the most common viral pathogens identified in paediatric cancer patients undergoing chemotherapy. Patients with pre-B acute lymphoblastic leukaemia and breast cancer who have undergone chemotherapy have reported cases of coronavirus infection. Cytotoxic therapies used in cancer chemotherapy such as 6MP and 6TG have shown to be specific inhibitors for SARS coronavirus in in vitro studies. However, further in vitro and in vivo studies are required to confirm this, especially in COVID-19. Currently, there is no scientific evidence of the interaction between methotrexate and Covid-19.

2. Low dose steroids and NSAIDs

Since the outbreak of the novel Covid-19 infection, various contradictory information has been circulated regarding the potentially negative effect of treating patients with non-steroidal anti-inflammatory drugs (NSAIDs), non-NSAIDs and corticosteroids. NSAIDs work through inhibition of the cyclooxygenase enzymes (COX-1/COX-2), which are involved in the synthesis of key biological mediators. These mediators in turn control inflammation. Corticosteroids are involved in a number of key physiological processes including the immune response and inflammation and low dose steroids are often prescribed to cancer patients with suppressed immune systems to prevent the development of related auto-immune diseases.

Results

A total of 58 studies were identified from the search terms, of which 13 were deemed suitable for inclusion (Supplementary Table 2). Our search did not identify any strong evidence for or against the use of ibuprofen for treatment of COVID-19 specifically. One

study did however link SARS-CoV to the downregulation of ACE2 which is upregulated by ibuprofen (12). The authors of this study were investigating the link between the severity of COVID-19 symptoms in patients with asthma and hypertension.

The only other study to investigate a non-steroidal anti-inflammatory drug was one which looked at indomethacin (13). This study suggested that indomethacin exhibited potent antiviral activity against canine coronavirus (dramatically inhibiting virus replication and protecting the host cell from virus-induced damage). This activity was also observed against human SARS-CoV at a concentration dose of 1mg/kg.

In general, there appeared to be a few positive results for the use of corticosteroids in viral infections such as SARS-CoV (14-19). Corticosteroids were widely used during the SARS-CoV outbreak due to their known ability to modulate a variety of involved cytokines (including IL-1, IL-6, IL-8, IL-12 and TNF-alpha) (14, 16). Various studies in humans noted that corticosteroids appeared effective in reducing immunopathological damage but concerns centred around the promotion of viral rebound and association with adverse events (including acute respiratory distress syndrome) (14). One laboratory study which treated porcine respiratory coronavirus infected pigs with dexamethasone suggested that 1 or 2 doses of the corticosteroid in the acute phase of infection may effectively alleviate early pro-inflammatory response, but prolonged administration may play a role in enhancing viral replication (18). A separate Chinese study, which separated SARS-CoV patients into 4 treatment groups, identified early high dose steroids in combination with a quinolone as producing the best patient outcomes (19). Nevertheless, one review stated that the WHO does not currently recommend corticosteroids in other viral diseases such as Dengue as the "glucocorticoid-mediated stimulation of the hypothalamic-pituitary-adrenal axis can also drive lymphocytopenia, or it may promote exaggerated pro-inflammatory responses that eventually cause a worsening of the pathogenic condition" (20).

Conclusion

The current literature does not give conclusive evidence for or against the use of NSAIDs in the treatment of COVID-19 patients, though there appears to be some evidence that corticosteroids may be beneficial in the treatment of SARS-CoV. However, it is important to note this is not specific to COVID-19.

3. TNF α blocker

The tumour necrosis factor (TNF) family of receptors and cytokines is very large and are often the targets for drugs. One example are TNF alpha (TNF α) inhibitors, which act by suppressing the physiologic response to TNF α . TNF α is a pro-inflammatory cytokine involved in autoimmune and immune-mediated disorders such as rheumatoid arthritis, ankylosing spondylitis, inflammatory bowel disease, psoriasis, hidradenitis suppurativa and refractory asthma. Inhibitors of TNF α may be used in their treatment.

Inhibition of TNF α can be achieved with a monoclonal antibody such as infliximab (Remicade), adalimumab (Humira), certolizumab pegol (Cimzia), and golimumab (Simponi), or with the receptor fusion protein etanercept (Enbrel).

Results

A total of three studies were identified, of which two were deemed suitable for inclusion (Supplementary Table 3). The first study was a research letter that suggested that TNF α has been implicated in the severe immune-based pulmonary injury caused by SARS coronavirus, suggesting that TNF α inhibitors could be a potential treatment for the acute respiratory disease syndrome caused by coronavirus (21).

The second study utilised 22 piglets to assess the efficacy of an anti-TNF α)therapy for endotoxin respiratory diseases and observed that TNF α blockade was not associated with decrease in disease severity (22).

Conclusion

Currently, there is no evidence indicating that TNF α blockade is harmful to patients in the context of Covid-19.

4. IL-6 blockade

Interleukin 6 (IL-6), promptly and transiently produced in response to infections and tissue injuries, contributes to host defence through the stimulation of acute phase responses, hematopoiesis, and immune reactions. Although its expression is strictly controlled by transcriptional and posttranscriptional mechanisms, dysregulated continual synthesis of IL-6 plays a pathological effect on chronic inflammation and autoimmunity (23). For this reason, tocilizumab, a humanized anti-IL-6 receptor antibody was developed. Other approved anti IL-6 drugs are siltuximab (Sylvant) and sarilumab. Several agents are in clinical trials: olokizumab (CDP6038), elsilimomab, BMS945429(ALD518), sirukumab (CNTO 136), CPSI-2364, ALX-0061, clazakizumab, olokizumab, sarilumab, sirukumab, ARGX-109, FE301 and FM101.

Results

A total of 108 studies were identified from the second search strategy, 23 were suitable for inclusion (Supplementary Table 4). The first search strategy found no hits.

IL6 an actor in the pathogenetic mechanisms of the coronavirus infection

- Covid-19 induces a pro-inflammatory generation and secretion of cytokines including interleukin (IL)-1b and IL-6 via the toll like receptors (TLR) that causes the production of active mature IL-1b which is a mediator of lung inflammation, fever and fibrosis. Anti-inflammatory cytokines, such as IL1-Ra, IL-37 or IL-38 could potentially provide relief in both systemic inflammation and fever occurring after infection (24).
- Cytokine profiles in patients diagnosed with SARS showed marked elevation of T-helper lymphocyte type 1 (Th1) cytokine interferon-gamma (IFN- γ), inflammatory cytokines IL-1 β , IL-6 and IL-12 for at least two weeks after disease onset. Children however presented a much milder cytokine and chemokine storm (16).
- The high levels of IL-6 in the acute stage associated with lung lesions found in SARS patients are activated by the viral nucleocapsid SARS-CoV N protein (25).
- Over induction of inflammatory cytokine and dysregulation of cytokine signalling has been observed in patients with SARS in comparison with other respiratory viruses including respiratory syncytial virus (RSV), influenza A virus (FluAV), and human parainfluenza virus type 2 (hPIV2). SARS-CoV and RSV induced high levels of IL-6 and RANTES compared with FluAV and hPIV2 (26).
- The N-protein of SARS-CoV induces pulmonary inflammatory reaction and acute lung injury, which were related to the increase and imbalance of pro-inflammatory and anti-inflammatory cytokines. Glucocorticoids could effectively alleviate the pulmonary inflammatory reaction induced by N-protein of SARS-CoV (15).
- SARS-CoV does not productively infect human macrophages (Mphi) or dendritic cells (DCs), however it modulates a massive release of IL-6 and IL-12 and compromises the endocytic capacity (e.g., antigen capture capture) of Mphi was significantly compromised (27).
- Changes in plasma T helper (Th) cell cytokines, inflammatory cytokines and chemokines in 20 patients diagnosed with SARS were assessed. The elevation of Th1 cytokine IFN- γ , inflammatory cytokines IL-1, IL-6 and IL-12 and chemokines IL-8, MCP-1 and IP-10 confirmed the activation of Th1 cell-mediated immunity and hyper-

innate inflammatory response in SARS through the accumulation of monocytes/macrophages and neutrophils (17).

IL-6 as a potential marker of disease severity in coronavirus infected patients:

- IL-6 blood measurements seem useful to diagnose severe Covid-19 cases. The findings suggest that IL-6 and D-Dimer level can be used to estimate the severity of COVID-19. The optimum critical point of IL-6 in the group was 24.3 pg/ml, which was the upper limit of no severe pneumonia. (28)
- The increased expression of IL-2R and IL-6 in serum is expected to predict the severity of the 2019-nCoV pneumonia and the prognosis of patients (29).
- The serum levels of IL-6 and CXCL-10 were significantly elevated in MERS-CoV patients who developed severe diseases. (30)
- A new lethal animal model was characterised for SARS-CoV. Strain v2163 had nine mutations that increased levels of IL-1alpha and IL-6 in mice. The high IL-6 expression was correlated with mortality (31).
- SARS vaccination was tested in a murine SARS model. A high level of IL-6 and on days 2 and 3 after SARS-CoV infection was closely linked to the virus replication and disease severity. (32)
- Interleukin-6 (IL-6) and IL-8 are key SARS-CoV-induced epithelial cytokines capable of inhibiting the T-cell-priming ability of dendritic cells, a cellular element of the host innate defenses against respiratory infections, leading to an exacerbated inflammatory cascades and severe tissue damage in SARS patients (33) .
- In patients with a diagnosis of SARS-associated coronavirus infection, there were no significant differences in peak levels of IL-6, IL-8 and TNF-alpha between patients with and without acute respiratory distress syndrome. However , CRP and TNF-alpha were associated with worse outcomes and might be used as prognostic markers of SARS (34).
- IFN-gamma, IL-18, TGF-beta, IL-6, IP-10, MCP-1, MIG were highly elevated in the acute phase sera of Taiwan SARS patients, being IL-18, IP-10, MIG, and MCP-1 were significantly higher in the death group than in the survival group. It suggests that an interferon-gamma-related cytokine storm was induced post SARS coronavirus infection (35).
- Eight patients with severe acute respiratory syndrome (36) were treated with ribavirin, which was not effective in reducing the SARS coronavirus load in three of eight. Elevated levels of interleukin (IL)-6 and IL-8 subsequent to the peak viral load were found in eight and six cases (37).
- A cytokine profiling was performed for 110 serum from healthy donors, patients with SARS, patients with severe SARS, and patients with SARS in convalescence. IL6 concentrations were significantly elevated in severe SARS patients, but the IL-6 concentrations were similar in convalescent patients and control subjects which suggested that IL6 is associated with SARS severity (38).
- The authors of this study set out to study the inflammatory cytokine profile in children with SARS. They found that the plasma concentrations' key proinflammatory cytokines, including IL-6, were not substantially increased in any of the patients throughout the course of illness. From this, the authors stated that the cytokine results cast doubt on the liberal use of corticosteroids in paediatric SARS patients (39)

Potential targets for therapeutic intervention related to IL6 regulation during infection

- ADAM-17 regulates IL-6 class switching as a mediator between pro- and anti-inflammatory responses to viral antigenic stimuli in Ebola, SARS-CoV and dengue infections in humans. Therefore, ADAM-17 should be considered as a potential target molecule for novel antiviral drug discovery for infections such as SARS-CoV (20)

- SARS-CoV ssRNA is a new therapeutic target given its capacity to cause acute lung injury in mice with a high mortality rate in vivo experiment suggesting that SARS-CoV specific GU-rich ssRNA plays a very important role in the cytokine storm associated with a dysregulation of the innate immunity (40).
- DUSP1 and p38 MAPK are potential therapeutic targets for coronavirus infectious bronchitis virus (IBV), given their capability to reduce the production of an excessive amount of IL-6 and IL-8 in the infected cells (41).

Potential therapeutic agents that inhibit the infection-induced production of IL6

- Pretreatment of primary cultures of human nasal and tracheal epithelial cells with glycopyrronium or formoterol decreased viral RNA levels and/or titers, the expression of the HCoV-229E and the infection-induced production of cytokines, including IL-6, IL-8, and IFN-beta. Treatment of the cells with the CD13 inhibitor 2'2'-dipyridyl decreased viral titers. Pretreatment of the cells with a combination of three drugs (glycopyrronium, formoterol, and budesonide) exerted additive inhibitory effects on viral titers and cytokine production (42).

Conclusion

It has been suggested that the pathogenesis of SARS-CoV is mediated by disproportional immune responses and the ability of the virus to circumvent innate immunity. The COVID-19 infection has also been observed to induce pro-inflammatory cytokine generation and secretion of cytokines such as IL-6, which dysregulates the local inflammatory responses that have been suggested as partially responsible for the devastating acute respiratory distress syndrome.

Moreover, it has been observed that COVID-19 induces high levels of IL-6 for at least two weeks after disease onset. Children presented lower levels of cytokine production. IL-6 has been suggested as a potential prognostic marker of Covid-19 disease severity. Different molecules related with the IL6 pathway have been suggested as potential therapeutic targets such as ADAM-17, SARS-CoV ssRNA, DUSP1 and p38 MAPK.

Currently, there is no scientific evidence of the beneficial impact of IL-6 inhibitors in the modulation of the COVID-19 infection. Further understanding of the role of IL-6 reduction will be forthcoming as the pandemic progresses and further clinical data becomes available. *In vitro* treatment with glycopyrronium, formoterol, and budesonide exerted additive inhibitory effects on viral titers and cytokine production human nasal and tracheal epithelial cells.

5. JAK inhibitors

Janus kinase (JAK) inhibitors work by inhibiting the activity of one or more of the Janus kinase family of enzymes including, JAK1, JAK2, JAK3, TYK3. JAKs interact with signal transducer and activator of transcription proteins (STATs) and the JAK-STAT pathway is central to cellular response to exogenous signals in the immune system. The JAK family of enzymes are responsible for signal transduction and JAK inhibitors play a major role in inhibiting and blocking cytokine release that can contribute to growth of malignant cells in cancer. JAK inhibitors are used in the treatment of cancer and inflammatory diseases such as rheumatoid arthritis (43).

This review focuses on how the JAK-STAT pathway can be manipulated to reduce viral entry and inflammation in patients with coronavirus. The main targets that the review highlighted were baricitinib (JAK inhibitor), IRE1 α (an endoplasmic reticulum stress sensor, leading to an increased expression of negative regulators of JAK-STAT SOCS1 and SOCS3) and combination therapies using tylophorine-based compounds with JAK2 inhibitors.

Results

Fifteen studies were yielded from the search of which 4 were deemed suitable for inclusion (Supplementary Table 5).

Baricitinib

Baricitinib (Olmiant) is a JAK inhibitor that was approved by the European Medicines Agency (EMA) in February 2017 for the treatment of moderate-to-severe active rheumatoid arthritis in adults with an inadequate response to one or more disease-modifying anti-rheumatic drugs (DMARDs) (44). One study suggested the use of Baricitinib to reduce the viral entry and inflammation caused by 2019-nCoV. Most viruses enter cells through receptor-mediated endocytosis. Angiotensin converting enzyme -2 (ACE2), a cell-surface protein expressed on cells in the kidney, blood vessels, heart, and alveola type 2 (AT2) cells in the pulmonary epithelia, may be the receptor that 2019-nCoV uses to infect lung cells. The authors of Richardson et al. (2020) suggest that by inhibiting adaptor associated protein kinase 1 (AAK1) receptor that promotes endocytosis involved in ACE2, Baricitinib may reduce both the viral entry and the inflammation in 2019-nCoV patients (45).

IRE1 α

Inositol-requiring transmembrane kinase/endoribonuclease 1 α (IRE1 α) is an endoplasmic reticulum stress sensor that leads to increased expression of negative regulators of JAK-STAT, suppressor of cytokine signalling (SOCS)-1 and SOCS-3 (46). Therefore, IRE1 α may be a novel target against coronavirus infection requiring further exploration.

Tylophorine-based compounds

Tylophorine-based compounds are isolated from plants and exert potent anti-coronaviral activities against SARS-CoV and MERS-CoV (47). Nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) activation is a common pro-inflammatory response of host cells to viral infection. Following *in vitro* analysis, Yang et al. (2017) suggests the use of a combination therapy for SARS-CoV or MERS-CoV, wherein a tylophorine compound known to target transmissible gastroenteritis virus (TGEV) and a JAK2 inhibitor synergise to block the alternative dominant NF- κ B activation mediated by JAK2 (48). Therefore, the combination treatment for the inhibition of coronavirus per se, e.g. viral genome replication, and blocking cellular NF- κ B activation by coronaviruses, is a promising approach for the development of anti-coronavirals.

Conclusion

Current studies suggest that although there are potential targets in the JAK-STAT pathway that can be manipulated in the treatment for coronaviruses, they are all in early stages and require further *in vitro* and *in vivo* studies to confirm their therapeutic effects.

6. IL-1 blockade

Interleukin-1 (IL-1) is a pro-inflammatory cytokine and an important mediator of local and systemic inflammation. Excessive IL-1 release during viral infections can cause lung and tissue inflammation, fever and fibrosis. IL-1 suppression has found to be effective in many inflammatory diseases including rheumatoid arthritis (24).

It is well established that an over-expression of interleukin-1 is a hallmark of SARS-CoV infection, probably through activation of transcription factor nuclear factor, activator protein 1 and activating factor 2. In COVID-19 specifically, the virus is thought to bind to toll like receptors (TLRs) which activate the formation of pro-IL-1 and activation of the inflammasome (24). This inflammasome activation is important for the regulation of cells of both the innate and adaptive immune system paving the way for specific immune responses. As part of the inflammasome activation, IL-1 β is subsequently produced which mediates the

inflammation of the lungs, fever and fibrosis thus causing respiratory complications in the infected host.

Results

A total of 37 studies were identified from the search of which 9 were deemed suitable for inclusion (Supplementary Table 6). Many of the studies were *in vitro* studies and repeatedly demonstrated increased IL-1 levels in patients infected with a coronavirus. One study investigated the levels of various inflammatory cytokines in 29, COVID-19 patients in China and compared the levels between general, severe and critically ill groups (49). The authors reported no significant differences in IL-1b levels between the three groups of patients. Another study involving 20 consecutive SARS patients admitted to a Hong Kong hospital identified significantly elevated levels of IL-1 β within the first 12, 7 and 5 days following onset of infection (17). Those patients with more severe disease were treated with pulsed methylprednisolone and IL-1 β levels returned to normal after 7 days. The 7 patients with less severe disease did not receive any dosage of corticosteroids and their cytokine levels returned to normal range levels over the same 7-day time period.

A further Chinese study also identified reduced levels of IL-1 β following administration of corticosteroids- suggesting inhibition of pro-inflammatory cytokines such as IL-1 may be a beneficial treatment strategy for treatment of SARS (50). A third study, which measured serum cytokine levels in 4 patient groups including controls, patients with SARS, patients with severe SARS and convalescent SARS patients suggested that longer term treatment (over a period of 7-10 days) with low-dose steroids can alter serum cytokine levels, including IL-1 α (38).

One rat model showed promising results for an IL-1 receptor antagonist which reduced the chemokine expression in infected animals (51). However, this result cannot be generalised for humans. Unfortunately, one study, in which the authors state the 'demonstrate for the first time that inflammation by coronavirus may be inhibited by anti-inflammatory cytokines belonging to the IL-1 family', was only available as an abstract (52). Therefore, further evidence or information to back this claim up is not available.

Conclusion

Overall, this review demonstrated that although it is evident that IL-1 is elevated in patients infected with a coronavirus, there is not at present evidence for an established role for IL-1 blockers in the treatment of COVID-19 in humans. The literature did, however, suggest a potential role for low dose corticosteroids to reduce levels of pro-inflammatory markers, such as IL-1, which are elevated as part of the immune response and may have a role in the severe lung damage associated with human coronaviruses.

7. Mycophenolate

Mycophenolate mofetil (which is a derivative of mycophenolic acid (MPA)) is an immune suppressant, antineoplastic and antiviral medication. According to the British National Formulary mycophenolate mofetil is used for the prophylaxis of acute rejection in renal transplantation and is usually used in combination with a corticosteroid and ciclosporin.

Results

Almost all of the studies investigated MPA as a potential therapy for Middle East Respiratory Syndrome Coronavirus (MERS-CoV) due to its anti-viral properties. Six of the 13 selected studies were *in vitro* studies, two were *in vivo*, one was a clinical example and four were reviews (therefore there was some overlapping of results) (Supplementary Table 7).

In-vitro studies

In general, the *in vitro* studies looked positive with MPA targeting the papain like proteases of both MERS-CoV and SARS-CoV (53, 54). The studies found that MPA showed strong inhibition of the virus with a very low IC50 (55-57).

In vivo study

MPA can be used in combination with interferon-beta (IFN- β). One study, which applied this regime in marmosets exhibiting a severe disease resembling human MERS, reported high viral loads with more severe or even fatal disease (28). The authors of this study state that MPA is likely to cause more harm than benefit to MERS patients.

Clinical studies

According to the review by Mo and Fisher (2016), MPA monotherapy had not been tested in a clinical setting for the treatment of MERS-CoV (58). Al Ghamdi et al (2016), presented an example where eight patients were treated with MPA for MERS-CoV, seven in combination with IFN- β (59). All eight of these patients survived, however the review by Mo and Fisher stated that this group of patients had lower Acute Physiology and Chronic Health Evaluation II (APACHEII) scores compared with others in the cohort who received a variety of antiviral agents including ribavirin and IFN- α , steroids and antibiotics. Therefore, the results must be interpreted with caution.

Conclusion

Whilst the *in vitro* studies showed promising results for MPA against MERS, the *in vivo* studies suggest that its use is likely to cause more harm than benefit and hence is not likely to be useful against coronavirus infections. The clinical studies are too small to confirm or deny any beneficial use for MERS-CoV patients.

8. Tacrolimus

Tacrolimus, also known as fujimycin, envarsus or FK506, is an immunosuppressive drug which is mainly administered after allogeneic organ transplant to lower risk of organ rejection. It's mechanism of action focusses on inhibition of calcineurin which is involved in the production of Interleukin-2 (IL-2). Interleukin-2 is a cytokine which promotes the development and proliferation of T cells which form a vital component of the human adaptive immune response.

Results

A total of 18 studies were identified from the search terms, of which three were deemed suitable for inclusion (Supplementary Table 8).

Overall, the literature appeared to suggest a potential role for tacrolimus in the treatment of human coronaviruses. In a case study of two renal transplant recipients who tested positive for MERS CoV, a patient who was being treated with an immunosuppressive regimen of tacrolimus underwent full recovery whilst the other patient (who was not on this treatment regimen) succumbed to the infection (60). The patient who eventually made a full recovery was also treated with antibacterial therapy and a reduced dose of mycophenolate mofetil and it is therefore not possible to conclude that patient recovery due to tacrolimus.

The two other included studies were both laboratory studies involving cell line culture. The first investigated pathways of coronavirus viral replication as potential antiviral therapeutic targets (61). Genome-wide SARS-CoV yeast-two-hybrid interaction screen with human cDNA libraries identified FK506-binding proteins as interaction partners of SARS-CoV non-structural protein 1. Subsequently, the authors investigated whether tacrolimus inhibits viral replication of human coronaviruses. VeroFM cells infected with SARS-CoV and other human

coronaviruses were treated with FK506. Results showed that FK506 effectively inhibited viral replication of SARS-CoV, HCoV-NL63 and HCoV-229E at non-toxic, low micromolar concentrations with a reduction in viral titers to undetectable levels. The second study further confirmed this inhibition using novel non-immunosuppressive derivatives of FK506 in the context of HCoV-NL63 at low micromolar, non-cytotoxic concentrations in cell culture (62).

Conclusion

Overall the small amount of literature available suggests a potential role of FK506 (tacrolimus) as a potent antiviral in the treatment of human coronaviruses. It is important to note, however, that COVID-19 is a novel disease and may have different aetiology and mechanistic action compared to existing strains and to date, this immunosuppressive drug and its derivatives has not been tested in humans. Further study is warranted, both in the clinical setting and laboratory.

9. Anti-CD20

No studies were identified for inclusion.

10. CTLA-4 Ig

No studies were identified for inclusion.

Overall conclusion

The rapidly progressing SARS-CoV-2 pandemic has led to challenging decision making about treatment of patients critically unwell with the novel viral infection. In parallel, doctors across multiple specialties are making clinical decisions about the appropriate continuation of treatments for patients with chronic illnesses requiring immune suppressive medication. This systematic review looks to provide guidance from the current available literature.

As the COVID-19 pandemic progresses collective effort to capture data from prospective trials is required. Sponsors of randomized controlled trials recruiting patients randomized to receive immune modulatory drugs that may be affected by COVID-19 should collect data about the disease outcomes and consider interim analysis of potential advantages and disadvantages associated with using one of these medications.

Low dose prednisolone and tacrolimus therapy may have beneficial impact on the course of the SARS-CoV-2. This observation requires further validation. The mycophenolate mofetil picture is less clear, with conflicting data from pre-clinical studies. There is no definitive evidence that specific cytotoxic drugs, low dose methotrexate for auto-immune disease, NSAIDs, JAK kinase inhibitors or anti- TNF α biological agents are contraindicated. There is evidence that IL-6 peak levels are associated with severity of pulmonary complications. Ongoing studies of blockade of the IL-6 pathway are rational and will hopefully inform practice as the pandemic progresses.

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