DR MARTIN BLOMBERG JENSEN (Orcid ID : 0000-0003-3800-4253)

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Cyclosporine and COVID-19: Risk or Favorable?

Nadia Nicholine Poulsen[1], Albrecht von Brunn[2], Mads Hornum[3,4], Martin Blomberg Jensen[1,5].

1) Group of Skeletal, Mineral, and Gonadal Endocrinology, Department of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark
2) Max von Pettenkofer-Institute, Ludwig-Maximilians-University Munich/German Center for Infection Research (DZIF), Partner Site Munich, 80336, Munich, Germany
3) Department of Nephrology, Rigshospitalet, Copenhagen, Denmark
4) Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark
5) Division of Bone and Mineral Research, HSDM/HMS Harvard University, Boston, MA, USA

ORCID

Martin Blomberg Jensen: https://orcid.org/0000-0003-3800-4253

Nadia Nicholine Poulsen: https://orcid.org/0000-0002-0625-1770

Mads Hornum: https://orcid.org/0000-0002-0123-4007

Corresponding author

Martin Blomberg Jensen: blombergjensen@gmail.com

Abbreviations ARDS: acute respiratory distress syndrome; COVID-19: coronavirus disease 2019; FKBP: FK506 binding protein; ICU, intensive care unit; IL-2: Interleukin-2; MERS-CoV: Middle East Respiratory Syndrome Coronavirus; NFAT: nuclear factor of activated T cells; Nsp1: Non-structural protein 1; RCT: randomized clinical trial; SARS-CoV: severe acute respiratory syndrome coronavirus; SARS-CoV2: severe acute respiratory syndrome coronavirus 2.

Abstract

The coronavirus disease 2019 (COVID-19) pandemic is declared a global health emergency. COVID-19 is triggered by a novel coronavirus: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2). Baseline characteristics of admitted patients with COVID-19 show that adiposity, diabetes and hypertension are risk factors for developing severe disease, but so far immunosuppressed patients that are listed as high-risk patients have not been more susceptible to severe COVID-19 than the rest of the population. Multiple clinical trials are currently being conducted, which hopefully can identify more drugs that can lower mortality, morbidity and burden on the society. Several independent studies have convincingly shown that cyclosporine inhibit replication of several different coronaviruses *in vitro*. The cyclosporine-analog Alisporivir has recently been shown to inhibit SARS-CoV2 *in vitro*. These findings are intriguing, although there is no clinical evidence for a protective effect to reduce the likelihood of severe COVID-19 or to treat the immune storm or adult respiratory distress syndrome (ARDS) that often causes severe morbidity. Here, we review the putative link between COVID-19 and cyclosporine, while we await more robust clinical data.

Introduction

In recent months the corona virus disease 2019 (COVID-19) pandemic has stressed health care systems worldwide and the World Health Organization has declared it a global health emergency. Patients treated with immunosuppressive treatment e.g. due to organ transplantation or autoimmune disease are instructed to isolate at home because of a presumed higher risk of more serious disease and possible death. COVID-19 is triggered by a novel coronavirus identified in December 2019 in Wuhan China and has been named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2). Two drugs have shown promising effect on COVID-19 patients, Remdesivir proved in a recent trial to reduce time to recovery, but had no effect on mortality[1]. Dexamethasone has in a preprint shown to reduce 28-day mortality in a subgroup of patients[2]. Dexamethasone and Remdesivir obviously targets COVID-19 differently, which highlights that the pathogenesis of the disease is complex. SARS-CoV2 induces a variety of symptoms such as fever, myalgia, dry cough, loss of smell and in some patients progress to a more severe disease requiring admittance to Intensive Care Units (ICU). The first clinical characteristics from China showed that a more severe disease outcome defined as need for ICU admittance or death was associated with presence of comorbidities particularly diabetes, obesity, hypertension, cardiovascular disease and chronic kidney disease[3–7]. There exist only very limited data on disease severity in transplanted patients treated with calcineurin inhibitors in combination with other drugs such as prednisolone or mycophenolate mofetil. However, the first sparse available data indicates that transplanted patients have no increased risk of severe disease despite of multiple and diverse comorbidities. Li et al[8] report of two cases of COVID-19 in heart transplant recipients. One had severe disease and both patients recovered. In two heart transplant recipients with COVID-19 in the US, one patient died from multiorgan failure and the other patient had mild disease and were discharged on day eight[9]. In four kidney transplant recipients from Wuhan, China it was stated that all patients benefitted from reduction of immunosuppressants, since all four patients had mild disease[10]. A 50 years old COVID-19 patient in Spain, treated with tacrolimus due to previous kidney transplantation needed treatment in the ICU on day 12[11]. These early case reports have now been supported by three cohort studies from transplanted patients in Europe[12–14]. In a study from Spain, 29 kidney transplant recipients with COVID-19 were evaluated. One group of patients (n=6) were reduced in calcineurin inhibitor dose and the second group (n=23) either continued their usual cyclosporine dose or were switched from tacrolimus to cyclosporine. The first group had a mortality of 50% and the second group only 12.5% thus supporting the idea that continuous use of cyclosporine might be beneficial in COVID-19 patients. However, the study is observational and not a blinded randomized clinical trial (RCT) and both groups were also treated with multiple non-protocolized drugs: two thirds of

the patients were given high dose steroid, one third were given intravenous immunoglobulin, one third an IL-6 inhibitor and all patients were given hydroxychloroquine, thus making it difficult to draw definite clinically conclusions from these interesting observations from Rodriguez-Cubillo and colleagues[15].

The transplantation society currently recommend close attention to patients with medication-induced lymphopenia, but no specific instructions on anti-rejection regimen exist due to current lack of evidence[16]. Romanelli et al.[17] have suggested that clinicians must consider to pause immunosuppressants in transplanted patients with COVID-19, which is a common strategy in transplanted patients with infections. This suggestion may in part be based on data from the epidemic of Middle East Respiratory Syndrome (MERS) as some case reports of kidney transplant recipients infected with MERS died[18,19].

The above-listed COVID-19 clinical observations may be premature, but they all indicate that immunosuppression and use of calcineurin inhibitors impose no increased risk for severe disease, and we speculate that calcineurin inhibitors may protect from severe disease and ultimately death because transplanted patients often have a high prevalence of other risk factors such as hypertension, diabetes and obesity. However, there may exist other explanations to why COVID-19 seems to be mitigated in transplant recipients e.g that this patient group is particular compliant to hygiene recommendations and preventive measures. Another explanation could be the easier access to hospitals and thus increasing numbers of detected COVID-19 infected with mild symptoms, which would otherwise not be found in the general population thus diluting the case fatality rate.

Computational methods looking into host-virus interactions and possible antiviral drug targets with repurposed drugs suggests tacrolimus among others as a potential drug against COVID-19[20,21]. A letter by Russel et al suggests that there is tantalizing *in vitro* evidence for cyclosporine as an anti-coronavirus agent as well as a potential disease-modifying role through inhibition of Severe Acute Respiratory Syndrome (SARS) coronavirus-mediated IL-2 induction and authors advocate that a trial of cyclosporine should be considered in the event of a future SARS epidemic[22]. These promising pilot data require appropriate power in larger studies before immunosuppression can be considered a low risk or maybe even protective for severe COVID-19 but here we review how cyclosporine may influence COVID-19.

COVID-19

Six coronaviruses have previously been shown to cause human disease but four of these are considered low pathogenic coronaviruses (229E, HKU1, OC43 and NL63) that causes mild upper respiratory tract infections[23], in contrast to the highly pathogenic β-Coronaviruses: Severe Acute Respiratory Syndrome-

CoV (SARS)[24,25] and Middle East Respiratory Syndrome CoV (MERS) that both cause severe lower airway infection and fatal viral pneumonia[23,26]. Back in 2002-2003 SARS infected more than 8000 causing at least 774 deaths with a case fatality rate of approximately 10%. MERS proved even more deadly causing around 2500 cases and approximately 900 deaths and a case fatality rate of approximately 35%[6]. Both SARS and MERS have a broad spectrum of symptoms ranging from flu-like symptoms to acute respiratory distress syndrome (ARDS)[27].

SARS-CoV2 is also a β-Coronavirus, it shares 79% homology with SARS[28,29] and has 96% sequence identity with bat coronavirus (BatCoV RaTG13)[29–31]. To understand the pathogenesis of COVID-19 it is important to discriminate between the tissue damage induced by the pathogen and the indirect and later effects caused by the immune response, which on one hand is required in order to eradicate the virus but also may induce significant organ damage. The main target for SARS-CoV2 is the lungs like SARS. Severe pneumonia develops and is often associated with massive inflammatory cell infiltration (lymphocytes, macrophages, neutrophils) and elevated proinflammatory cytokine/chemokine responses resulting in acute lung injury and ARDS[23]. The incubation period of SARS-CoV2 is typically 2-7 days[32] before the patients present with fever, cough, dyspnea, fatigue and myalgia[4,32–34], which may be accompanied by rhinorrhoea, pharyngalgia, anosmia, ageusia and diarrhea[6]. The median age of hospitalized patients has been reported to be around 49-63 years old in China[4,7,34–36]. A remarkable high number of patients below 70 years of age without significant comorbidities are requiring admittance in the ICU with ventilator support, with a median age of 61 years in the United Kingdom (UK)[37]. A recent study showed that of patients admitted to ICU 61.1% had ARDS, 44.4% had arrythmia and 30.6% had shock[35]. The high frequency of ARDS may be the cause for admittance to ICU but other studies investigating hospitalized patients with no ICU admittance report ARDS in 17-29% of all hospitalized patients[33,34] and a case fatality rate of 3.6-6.8%[33,38]. Early data from China showed a mortality rate of 11-16% among hospitalized patients[4,7,34]. The late but typically abrupt onset of severe COVID-19 with hypoxemia and dyspnea requires hospital admission and some rapidly progressed to ARDS that eventually would lead to septic shock, metabolic acidosis, coagulation dysfunction, and multiple organ dysfunction syndrome[6,32,35]. It remains to be shown if these symptoms are similar or reduced in immunocompromised patients due to inhibition of the cytokine response as previously shown for parainfluenza virus[39]. Data from 21 solid organ transplant recipients from Switzerland showed almost similar clinical presentation, however, remarkably the severity and complication rates were not higher than in the general population with 19% developing ARDS, 9.5% died and 23% needed ICU admission[13]. Another Spanish study including 33 kidney transplant recipients showed that 52% were admitted to ICU, 6% needed mechanical ventilation and 6% died[12]. The study

concluded that the severity of COVID-19 was not different in immune compromised patients. Although these findings are in opposition to a small American cohort study reporting more severe outcome in transplant recipients with 34% ICU admission and 24% death of hospitalized cases[40].

Severity of COVID-19 is related to the onset of lower respiratory tract disease and pneumonia that typically occur 7-10 days after onset. This seems slightly delayed compared with influenza virus pneumonia[32,41,42]. Lymphocytopenia has been suggested to be a negative predictor of disease progression and prognosis, while newer data suggest that elevated D-dimer, CRP, viral load and low albumin are poor prognostic signs. None of these markers have been reproduced consistently and they all need to be validated in larger ongoing clinical trials. The most common finding on CT scan was bilateral pneumonia and ground-glass opacities[33] that were associated with more severe disease manifestations. Other radiological findings such as interlobular septal thickening, pulmonary consolidations and the so called white lung due to atelectasis and pleural effusion were also associated with severe COVID-19[32,43]. A comprehensive Chinese study showed that older age and lower CD4-T-cell count were associated with ICU-admission and ARDS, and highly elevated interleukin-6 (IL-6) levels in ICU patients was suggestive of a cytokine storm and an inappropriate host-inflammatory response in patients with severe disease[36]. Indeed, patients admitted to the ICU had higher levels of numerous cytokines when compared to patients not in the ICU[4]. It has been speculated that the reason why children has less severe disease during this pandemic is because of the role of the thymus gland and thus the difference in cytokine expression[44]. On the other hand, the overactive immune reaction may be due to impaired viral clearance again highlighting that appropriate immune system activity is required at all times. Postmortem studies of COVID-19 patients showed diffuse alveolar damage, cellular fibromyxoid exudates, desquamation of pneumocytes and hyaline membranes in the lung equivalent to ARDS[45,46]. They also report of a hyperactive state with overactivated T-cells and suggest that most of the lung injury seems to be caused by severe immune injury.

Calcineurin inhibitors and coronavirus replication

The outbreak of SARS, MERS and now COVID-19 has demonstrated human vulnerability to coronavirus epidemics. Neither vaccines nor therapeutics are available against human or animal coronaviruses and most of the drugs used in ongoing clinical trials to treat COVID-19 have been selected because they repress coronavirus replication *in vitro*. One of the consequences of the SARS epidemic was an increase in

efforts to understand coronavirus replication and identify additional possible targets for anti-viral therapy including calcineurin[47]. Pfefferle et al investigated coronavirus-host interactions using a genome-wide yeast-two hybrid screening and identified the coronavirus non-structural protein 1 (Nsp-1) and several immunophilins as important in virus-host response[48] and Luo et al[49] report that the nucleocapsid protein of SARS-CoV bind to cyclophilin A.

Cyclosporine and tacrolimus are the most used calcineurin inhibitors in daily clinical practice for prevention of alloimmune response in transplantation[50,51]. Both compounds suppress the immune system and the main action is prevention of interleukin-2 (IL-2) production in T cells[52]. Cyclosporine and tacrolimus are chemically distinct molecules that bind to the intracellular immunophilins cyclophilin[52–55] and FK506 binding protein (FKBP)-12[56], respectively, and this calcineurin-inhibitor-immunophilin complex inhibits the phosphatase activity of calcineurin which regulates nuclear translocation and subsequent the activation of nuclear factor of activated T cells (NFAT). NFAT inhibition will eventually lead to the block of transcription of cytokines and thus inhibition of T cell activation[48,52–55,57–59] (figure 1).

Several independent studies have shown that coronavirus replication and growth depend on active immunophilin pathways. This has been shown for several human coronaviruses including HCoV-NL63[60,61] and HCoV-229E[62,63]. However, studies silencing specific immunophilins only show partial effect thus indicating a more complex mechanism of action[53,64,65]. The immunophilin pathway is inhibited by both tacrolimus and cyclosporine. Cyclosporine at non-cytotoxic concentrations induces a strong inhibition of the replication of several coronaviruses including SARS-CoV[48,64] (EC_{50} 3.3 µM), MERS-CoV[66] and HCoV-229E *in vitro*[64]. Cyclosporine alone or in combination with interferon alpha also inhibit replication of MERS in human *ex vivo* explant cultures[67]. Tacrolimus ina low non-cytotoxic concentrations inhibits the growth of numerous human coronaviruses including SARS-CoV (EC_{50} 6.9 µM) *in vitro*[60]. Moreover, several cyclosporine- and tacrolimus analogs, which are pharmaceutical agents similar to either cyclosporine or tacrolimus but designed to lack the immunosuppressive effect, including the drug Alisporivir, have demonstrated potent suppressive effects on replication of multiple coronaviruses *in vitro* such as SARS-CoV (EC_{50} 1.3-8.3 µM)[68], MERS-CoV (EC_{50} 1.5-4.0 µM)[68], HCoV-NL63 (EC_{50} 0.8 µM)[61] and HCoV-229E (EC_{50} 1.37-2.77 µM)[62], and now two independent studies have found that Alisporivir inhibits SARS-CoV2 *in vitro* with EC_{50} of 4.9 µM[69] and 0.46 µM[70], respectively. Alisporivir was tested *in vivo* but was unable to diminish morbidity or mortality in an *in vivo* mouse model, which highlights that *in vitro* findings have a hard time being extrapolated to the clinical setting[68]. Noteworthy, cyclosporine and derivates have also *in vivo* been shown to be effective against other virus such as influenzavirus[71], HIV and Hepatitis C Virus and usage in clinical studies of Hepatitis-C-infected adults showed promising results[72–75], but has not had any clinical

implications and warrants further studies. Still the *in vivo* effect and particularly clinical effects of calcineurin inhibitors on SARS-CoV2 have yet to be confirmed.

The *in vitro* findings on coronavirus replication and beneficial effects in other virus diseases *in vivo* are intriguing, but the obvious question is whether the dosage of cyclosporine required to attain efficient inhibition of SARS-CoV2 in the lungs is safe and tolerable. The cyclosporine concentration required to inhibit virus replication exceeds by far the serum concentrations that typically are well below 200 ng/mL[76–78]. This implies that the dosage used to treat most patients with cyclosporine is too low to effectively eradicate the virus. One of the challenges is to obtain sufficient tissue concentration, as the main virus load is in the airways and lungs and not in serum and the concentration of cyclosporine in the lungs is lower than in serum. Moreover, the required dosage for actively treating patients with severe COVID-19 would be 3-6 fold higher, which in turn would cause severe adverse and possible toxic effects especially nephrotoxicity[79,80]. Moreover, the free available fraction and particularly the local tissue concentration of cyclosporine in the lungs would be too low to induce a substantial inhibition of virus replication. Peak concentration in serum cyclosporine could in theory reach levels that approximate antiviral concentrations but the only way to reach high local tissue concentrations would be through cyclosporine inhalation. Inhaled cyclosporine has been tested in animals[81], healthy volunteers and lung transplant recipients[82,83] and the lung concentration of inhaled cyclosporine is three times higher than when systemically administered[81]. It is generally well tolerated although a few cases of transient reduced forced expiratory volume in the first second (FEV1) following inhalation, has been reported. Inhaled cyclosporine is not available as routine treatment and cannot be advised at this moment for COVID-19 patients as there is no human *in vivo* proof of an antiviral effect.

The Immunonephrology Working Group of the European Renal Association–European Dialysis and Transplant Association has published recommendations for the management of patients with immune-mediated kidney disease during this current pandemic, and authors point out that patients with mild COVID-19 might continue low dose of cyclosporine due to the *in vitro* evidence of inhibition of coronavirus replication[84]. Moreover, a recent comment supports the idea that cyclosporine might be the drug-of-choice during the COVID-19 pandemic for kidney transplant recipient due to the *in vitro* evidence and thus providing an “old” alternative to the routine rejection regimens[85]. However, rejection rates are higher in patients on cyclosporine compared to tacrolimus in kidney[86], heart[87] and liver transplant recipients[88]. The defining evidence would be to investigate cyclosporine treatment in hospitalized patients with COVID-19. There is reason to believe that it would be safe to treat this patient group with low dose cyclosporine (<3 mg/kg). Previous studies have shown, that cyclosporine is safe to use in critically ill patients with severe infections, inflammatory diseases and even circulatory vulnerable patients, and high-

dose cyclosporine (4.5–8.3 mg/kg) is well tolerated in steroid-refractory ulcerative colitis[89]. Moreover, a meta-analysis showed that cyclosporine had a beneficial effect on mortality for Stevens-Johnson syndrome and toxic epidermal necrolysis[90] where rapid onset of treatment response as in COVID-19 is warranted. In a randomized, double-blinded, placebo-controlled trial cyclosporine improved lung function when given to severe asthma patients for 3 months and significantly lowered exacerbation rate compared to the placebo group and was well tolerated in patients with severe asthma[91]. In a multicenter, double-blind, randomized trial of bolus injection of cyclosporine was tested in patients with an acute anterior ST-segment elevation myocardial infarction (STEMI) who were undergoing primary percutaneous coronary intervention. Cyclosporine did not result in improved clinical outcomes compared to placebo, however interestingly the authors did not find any significant difference in the safety profile between the two treatment groups thus indicating that cyclosporine treatment in these circulatory unstable patients was well tolerated[92]. In a randomized controlled trial of cyclosporine plus intravenous immunoglobulin (IVIG) treatment or IVIG alone to children with Kawasaki disease found that combined treatment with cyclosporine and IVIG reduced the incidence of coronary artery abnormalities. Authors report no difference of adverse effects in the two groups and concludes that cyclosporine treatment is safe and well tolerated[93]. This is of particular interest due to the observed increased incidence of Kawasaki disease during the COVID-19 pandemic[94]. One case report from Japan of a 10 year old boy with a mycoplasma pneumoniae lung abscess with laboratory indication of cytokine storm was treated with cyclosporine which suppressed the hypercytokinemia[95]. Based on the presented findings, all of these studies show that cyclosporine is safe to give to a broad range of critical ill patients and we believe that it is safe to investigate cyclosporine in a placebo or dexamethasone controlled trial of COVID-19 patients requiring admittance to hospital.

We cannot recommend switching anti-rejection regimen during COVID-19, as the available data reviewed here is not sufficient to recommend replacing tacrolimus with cyclosporine during severe COVID-19. However, we do suggest that revised guidelines should recommend continuing cyclosporine to patients during COVID-19 except in cases of renal failure, severe leucopenia or high serum cyclosporine levels. A switch from tacrolimus to cyclosporine would at this point be based purely on positive observational data with a putative benefit for COVID-19 morbidity but with a possible higher risk of rejection and we warrant controlled studies to test whether this switch is advisable or not.

Conclusion

Remdesivir and dexamethasone are the only drugs available with proven effect on COVID-19, although more efficient therapy is warranted and most patients are still only receiving supportive care including oxygen and empirical antibiotic therapy to prevent secondary infections. Currently, more than 200 new clinical trials are registered at clinicaltrials.org testing various treatments e.g. ACE-inhibitors, serine protease inhibitors, IL-6 inhibitors, JAK-inhibitors, interferons, antivirals or azithromycin. To the best of our knowledge no clinical controlled trial is being conducted to test the effect of any calcineurin inhibitor. Several groups have found *in vitro* evidence of cyclosporine mediated inhibition of replication of several coronaviruses including Severe Acute Respiratory Syndrome-CoV (SARS) and Middle East Respiratory Syndrome CoV (MERS) and the cyclosporine-analog Alisporivir inhibits SARS-CoV2 *in vitro*. Due to the limited number of patients and quickly contained SARS and MERS epidemics, these compounds have never been tested in a clinical setting before. We are still awaiting robust data from COVID-19 patients actively treated with calcineurin inhibitors due to transplantation or autoimmune diseases but so far there is no evidence that use of cyclosporine possess an additional risk for severe COVID-19 in addition to the co-morbidities such as diabetes, smoking, hypertension and obesity that often co-exist in these patients. More controversial but not less intriguing is the putative impact of cyclosporine on severe COVID-19, which ultimately should be tested in a RCT in hospitalized patients during this current pandemic to determine whether cyclosporine could reduce the need for ICU admittance and high oxygen demand.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the American Journal of Transplantation.

Figure legends

Figure 1: Overview of the two most commonly used calcineurin inhibitors and the possible effects on COVID-19. FKBP: FK506 binding protein; IL-2: Interleukin-2; NFAT: nuclear factor of activated T cells; Nsp1: Non-structural protein 1.

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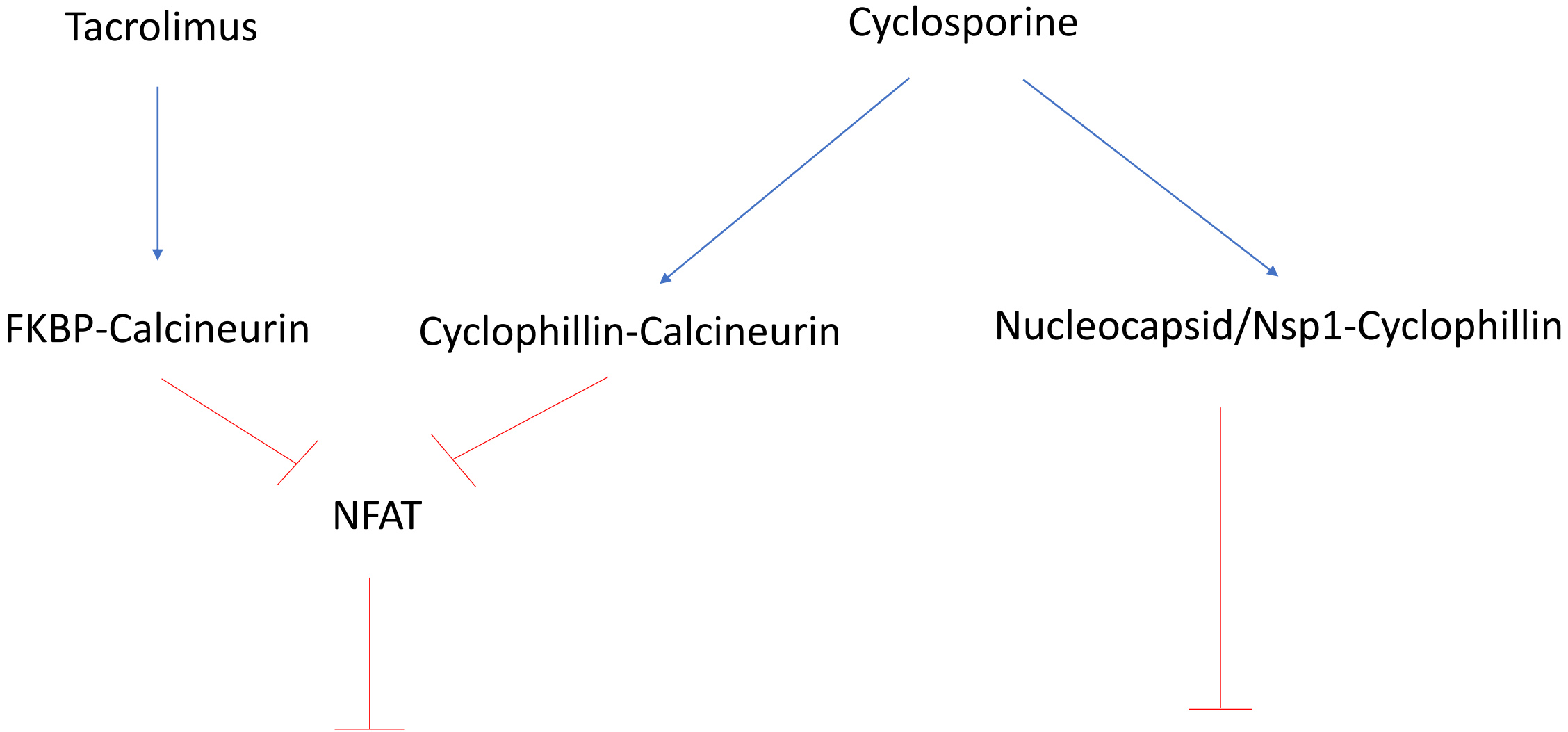
Tacrolimus
Cyclosporine
FKBP-Calcineurin
Cyclophillin-Calcineurin
Nucleocapsid/Nsp1-Cyclophillin
NFAT
Transcription of e.g. IL-2 in T-cells
Coronavirus replication