Immunosuppression for liver transplant recipients during the COVID-19 pandemic

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Received October 5, 2021

Revised October 24, 2021

Accepted October 25, 2021

Published online November 2, 2021

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SARS-CoV-2 infection has resulted in a pandemic with serious consequences for worldwide health care system. Liver transplant recipients manifest immunosuppressed status and are theoretically more vulnerable to infection, suggesting the need to modify the immunosuppressive regimens according to the patient status and the treatments used. This study intended to present the action mechanisms of immunosuppressive agents and show the balance between immunosuppressive effects and antiviral agents in relation to immunosuppressive treatment in patients with COVID-19 infection. In the absence of robust evidence to support general recommendations, experience with other viral infections suggests the benefit of management of immunosuppression without mycophenolate mofetil or mammalian target of rapamycin inhibitors. It is also important to consider the possible drug interactions, especially in the case of tacrolimus, with a few antiviral treatments in the context of COVID-19. The immunosuppressive effect of immunomodulating drugs administered to patients with severe lung disease also should be taken into account. The present study reviews the mechanisms of action of the different immunosuppressive agents, as well as their potential effect on SARS-CoV-2 infection. Guidelines for the management of immunosuppression in liver transplant recipients are suggested.

Keywords: SARS-CoV-2; Immunosuppression; Immunomodulation; Cytokines; Pneumonia; Liver transplantation

INTRODUCTION

Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus has generated a pandemic with serious health and economic crisis. Every effort has been made to fight this disease to ensure survival of patients [1-10]. Liver transplantation (LT) recipients are immunosuppressed and thus theoretically more vulnerable to infection, suggesting the need to modify the immunosuppressive

regimen according to the patient status and the treatments used. This study is intended to present the mechanisms of action of immunosuppressive agents and demonstrate the balance between immunosuppressive and antiviral effects in patients with COVID-19.

SARS-CoV-2 Virus

SARS-CoV-2 virus belongs to family Coronaviridae, which includes 4 subtypes of alpha, beta, gamma, and

delta. Coronaviruses infecting humans belong to the alpha (HCoV-229E and HCoV-NL63) and beta (SARS-CoV, MERS-CoV, HCoV-OC43 and HCoV-HKU1) subtypes that are known to cause lower respiratory tract infections and therefore associated with severe clinical conditions (SARS-CoV, MERS-CoV and SARS-CoV-2). Similar to other subtypes in the family, SARS-CoV-2 is a positive-stranded RNA virus, which encodes structural and non-structural proteins in different open reading frames [11-13].

The primary source of SARS-CoV-2 spread is human-to-human transmission through close contact or from fomites when a patient coughs. It has a high infectivity, colonizing the organism through the respiratory tract and especially infecting the alveolar epithelial cells [14,15]. Its replication is rapid and the infection is generalized, but most patients are asymptomatic or have only mild symptoms [12]. There are three phases of infection. The first is asymptomatic phase with a high viral load; the second is mild symptomatic phase, with dry cough, odynophagia, anosmia, fever, fatigue and occasionally diarrhea; and the third phase is characterized by severe cases, producing pneumonia with respiratory failure and dyspnea, which can lead to adult respiratory distress syndrome and finally to multi-organ failure and death [12,15,16]. In this third phase, viremia can be lowered and multiple organs are involved due to cytokine storm, which results from the exaggerated response of the immune system to SARS-CoV-2 infection [17].

THE IMMUNE SYSTEM IN RESPONSE TO SARS-COV-2

Innate Response

The presence of lymphopenia combined with neutrophilia, high levels of IL-6, increased C-reactive protein, and high levels of cytokines associated with innate immunity such as interferon gamma-induced protein-10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 α (MIP-1 α) and tumor necrosis factor- α (TNF α) suggest that it plays a very important role in the inflammatory response associated with SARS-CoV-2 infection [17-21].

The innate immune effector response against viruses is mainly based on interferon-1 (IFN-1) [19]. Thus, the cells of the innate immune response must recognize the invasion of the virus based on the molecular patterns associated with pathogens (PAMPs) [19]. This recognition leads to the activation of IFN-1 and other pro-inflammatory cytokines [20,21]. Blocking the interferon pathways facilitates the

replication of the virus and the release of additional pro-inflammatory cytokines that induce an excessive response [22].

Adaptive Response

In general, the Th1-type immune response plays a predominant role in viral infections [18,21,23]. Cytokines generated by antigen-presenting cells direct the response of T cells: regulatory T cells in general coordinate the adaptive response, whereas cytotoxic T cells are essential for viral elimination. The humoral immune response plays a role in limiting infection and preventing reinfection through production of neutralizing antibodies [23-29].

IMMUNOSUPPRESSION IN LIVER TRANSPLANTATION AND COVID-19

Immunosuppression in LT, although using the same drugs as those used in other solid organ transplants, is characterized by a lower intensity of immunosuppression [30-33]. This feature may lead to temporary withdrawal of some immunosuppressants that are used, for example, in severe infection, kidney failure, neurological disorders and others [30,31]. The immune response plays a very important role in COVID-19, thus the common immunosuppressive agents have two sides facilitating infection first and then attenuating the inflammatory response. In LT, as in other solid organ transplantations, immunosuppressive treatment is achieved using a calcineurin inhibitor (CNI), cyclosporine or tacrolimus. In addition, mycophenolate mofetil (MMF) is used in many patients with renal failure or for prevention. A mammalian target of rapamycin (mTOR inhibitor), such as everolimus, is less frequently used for calcineurin-sparing or antitumor effect.

In the context of COVID-19 infection in LT recipients, it is important to review the immunosuppressive regimens. The potential interaction of immunosuppressants with antiviral or antibacterial drugs used in the early stages of SARS-CoV-2 infection (lopinavir/ritonavir, azithromycin) requires special attention. Likewise, the interaction with other immunomodulators directed directly at the cytokine response (anti-IL6, anti-IL1), used in the treatment of severe patients COVID-19 [22,34,35]. Administration of these agents should be taken into account when adjusting the doses of immunosuppressants to avoid the risk of excessive immunosuppression.

Calcineurin Inhibitors

Cyclosporin or tacrolimus are potent inhibitors of lymphocyte calcineurin phosphatase activity and are considered the basic drugs for immunosuppression in LT [32]. Currently, these CNIs are often used in combination with MMF or everolimus in order to maintain lower plasma levels and avoid adverse side effects [30,31]. Cyclosporin A binds to cyclophilin and forms an active complex that inhibits the phosphatase activity of calcineurin. Calcineurin dephosphorylates the cytoplasmic component of the nuclear factor of activated T lymphocytes (NFATc), to reach the nucleus and activate the genes involved in the synthesis of IL-2, IFN- γ , IL-4, and TNF- β 46. Thus, cyclosporin A, by inhibiting calcineurin, inhibits T cell proliferation to prevent the clonal expansion of helper and cytotoxic T cells [36]. Tacrolimus acts similarly, but binds to a specific immunophilin (FKBP) to block the phosphatase activity of calcineurin and thus inhibit the transcription of genes involved in IL-2 synthesis. Its net effect on COVID-19 and particularly on SARS-CoV-2 is unknown. However, data indicate a direct antiviral effect of CNIs [36]. Such antiviral effect of CNIs, mainly cyclosporin A, was already suggested in the case of other viruses such as hepatitis C virus [37]. However, the balance between antiviral and immunosuppressive effects does not allow to observe a net antiviral effect. The information currently available does not allow us to affirm that CNIs have a significant positive or negative effect on COVID-19.

When simply dividing the patients with COVID-19, they can be classified into two forms: The mild form includes asymptomatic cases or symptomatic cases manifesting fever, dry cough, fatigue, and other symptoms, but without respiratory failure; and the severe form includes patients with pneumonia and respiratory failure. In mild patients, the recommendation is not to modify CNI, unless antiviral agents such as ritonavir-lopinavir are used with high interference, particularly with tacrolimus [38]. In such a case, the tacrolimus dosage can be reduced to 0.5 mg every 3–5 days. Obviously, this requires intensive control of the tacrolimus levels. Interactions with other treatments (azithromycin, hydroxychloroquine and others), which do not require an initial dose decrease, but require controlled levels of CNIs. In the case of seriously ill patients, it is better to reduce or withdraw CNIs.

Mycophenolate Mofetil

MMF is a prodrug that is converted to mycophenolic acid (MPA) in the body. MPA is mainly metabolized via glucuronidation by the enzyme uridine 5'-diphosphate-gluc-

ronosyltransferase [33]. MPA undergoes enterohepatic recirculation, which lengthens its half-life. MPA is a reversible and non-competitive inhibitor of inosine-5'-monophosphate dehydrogenase (IMPDH). It inhibits the proliferation of T and B lymphocytes and the production of immunoglobulins via depletion of the guanosine and deoxyguanosine pool in the lymphocytes [33,38]. In addition to this potent immunosuppressive action, MPA has broad in vitro and in vivo activity in animal models against various viruses [39-42]. MPA is also capable of inhibiting hepatitis C virus replication in vitro and in vivo by increasing the gene expression of interferon and by depleting guanosine [43]. In relation to coronaviruses, MPA was not effective against SARS-CoV in an animal model, but it was effective against MERS-CoV [44].

In contrast to these antiviral effects in vitro and in animal models, the reality in the clinical practice of LT is very different, in which the immunosuppressive effect clearly predominates, as seen with hepatitis C virus and cytomegalovirus. Therefore, MMF should be withdrawn if an LT recipient has SARS-CoV-2 infection at any stage or severity of COVID-19. Regardless of its known immunosuppressive effect, it can cause leukopenia, lymphopenia, thrombocytopenia and medullary aplasia, complications that can make the resolution of the infection extremely difficult or threaten the patient's life.

mTOR Inhibitors: Sirolimus or Everolimus

Sirolimus is a macrolide derived from the actinomycete *Streptomyces Hygroscopicus* discovered on Easter Island [45]. It is metabolized by cytochrome p450 3A4 requiring adjustment of plasma levels. Ninety percent of its metabolites are excreted in the feces. Action of mTOR inhibitors requires complex formation with an immunophilin. Similar to tacrolimus, they bind to FKBP-12, but in contrast, they do not inhibit calcineurin but rather the mTOR, which leads to abrogation of the signal from the IL-2 receptor, thereby inhibiting the proliferation of T and B37 cells. Everolimus is a derivative of sirolimus with a shorter elimination half-life and greater oral bioavailability.

The mTOR inhibitors exhibit antiviral properties in addition to their immunosuppressive and antiproliferative effects, which are well demonstrated in transplant patients in terms of prevention and treatment of infections caused by cytomegalovirus or BK62 virus. Despite suggestions of antiviral effect against coronaviruses via the pathways associated with the mTOR complexes 1 and 2 together with the activation of protein kinase (AMPK), the evidence is

scarce. Only a single study involving patients with pneumonia and respiratory failure due to influenza virus reported a shorter duration of symptoms and a lower incidence of multi-organ failure in patients who received rapamycin [46]. There is no clinical evidence supporting or recommending its use in COVID-19 infection. Drug interactions with other medications and the potential for associated leukopenia and lymphopenia should be considered.

RECOMMENDATION FOR ACTION IN LT RECIPIENTS AND COVID-19

The following recommendation is proposed by a Spanish transplant group [47]. This proposal aims to provide a few general lines of action that require modification for treatment of patients. This proposal is based on a very simple classification, which enables to alter immunosuppressive treatment before the development of complications associated with it.

For LT recipients with asymptomatic or mild COVID-19 infection (dry cough, odynophagia, anosmia, fever, fatigue and sometimes diarrhea): 1) Suspend or reduce MMF or everolimus if they are part of the treatment whenever possible, replacing them with low doses of prednisone; 2) Thoroughly monitor drug interactions, especially in patients undergoing treatment with CNIs receiving antivirals.

For LT recipients with severe infection (pneumonia in any of its stages): 1) Stop MMF or everolimus; 2) Reduce or suspend CNIs and replace with corticosteroids; 3) Take into account whether the patient has received immunomodulators treatments (such as IL-6 receptor inhibitors) when deciding the dose of corticosteroids; 4) Monitor drug interactions closely, especially in patients receiving antivirals.

FUNDING

There was no funding related to this study.

CONFLICT OF INTEREST

All authors have no conflicts of interest to declare.

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Conceptualization: All. Data curation: All. Project administration: JIP. Validation: All. Writing - original draft: All. Writing - review & editing: All.

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