Title: COVID-19 Vaccines and the Virus: Impact on Drug Metabolism and Pharmacokinetics

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ABSTRACT

This article reports on an American Society of Pharmacology and Therapeutics, Division of Drug
Metabolism and Disposition symposium held at Experimental Biology on April 2nd, 2022, in
Philadelphia. As of July 2022, over 500 million people have been infected with SARS-CoV-2
(the virus causing COVID-19) and over 12,000,000,000 vaccine doses have been administered.
Clinically significant interactions between viral infections and hepatic drug metabolism were first
recognized over 40 years ago during a cluster of pediatric theophylline toxicity cases attributed to
reduced hepatic drug metabolism amidst an influenza B outbreak. Today, a substantive body of
research supports that the activated innate immune response generally decreases hepatic
cytochrome P450 (CYP) activity. The interactions extend to drug transporters and other organs
and have the potential to impact drug absorption, distribution, metabolism, and excretion
(ADME). Based on this knowledge, altered ADME is predicted with SARS-CoV-2 infection or
vaccination. The report begins with a clinical case exploring the possibility of SARS-CoV-2
vaccination increasing clozapine levels. This is followed by discussions of how SARS-CoV-2
infection or vaccines alter the metabolism and disposition of complex drugs, such as
nanoparticles and biologics and small molecule therapies. The review concludes with a
discussion of the effects of viral infections on placental amino acid transport and their potential to
impact fetal development. The session improved our understanding of the impact of emerging
viral infections and vaccine technologies on drug metabolism and disposition, which will help
mitigate drug toxicity and improve drug and vaccine safety and effectiveness.

SIGNIFICANCE STATEMENT

Altered pharmacokinetics of small molecule and complex molecule drugs and fetal brain distribution of amino acids following SARS-CoV-2 infection or immunization are possible. The proposed mechanisms involve decreased liver CYP metabolism of small molecules, enhanced innate immune system metabolism of complex molecules and altered placental and fetal blood-brain barrier amino acid transport, respectively. Future research is needed to understand the effects of these interactions on adverse drug responses, drug and vaccine safety and effectiveness and fetal neurodevelopment.

Introduction

Historical perspectives. Early observations of interactions between infectious diseases and drug

metabolism and disposition date to the 1960s and 70s. These observations included altered

quinine metabolism during malaria infection, increased theophylline half-life in children with

influenza infection or adenoviral respiratory tract infections and increased cerebrospinal fluid

accumulation of ethambutol and rifampin in tuberculosis meningitis (Brooks et al., 1969; Place et

al., 1969; Sippel et al., 1974; Chang et al., 1978). Around the same time period, pioneering work

completed by Renton and Mannering discovered that interferon-inducing agents decreased

hepatic cytochrome P450 (CYP) activities, paving the way for the idea that inflammatory

cytokines are a regulatory link between infectious or inflammatory diseases and hepatic drug

metabolism (Renton and Mannering, 1976a; Renton and Mannering, 1976b). Shortly thereafter,

the clinical significance of interactions between infections and hepatic drug metabolism were

established during a cluster of pediatric theophylline toxicity cases attributed to reduced hepatic

CYP1A metabolism amidst an influenza B outbreak in 1980 in Seattle, Washington (Kraemer et

al., 1982).

In the ensuing years there have been many clinical studies and case reports in adult humans

supporting that infections or inflammation downregulate hepatic cytochrome P450 enzymes,

although increases in activity have also been reported in some instances (as recently reviewed

(Goralski et al., 2018; Lenoir et al., 2021c)). The observed effects on hepatic CYP metabolism

are dependent on the type of inflammatory or infectious stimuli and the specific CYP enzyme

(Goralski et al., 2018; Lenoir et al., 2021c). The metabolism of CYP3A4, CYP2C19 and

CYP1A2 substrates appears to be most affected with lesser or no impact on CYP2C9 and

CYP2D6 substrates (Lenoir et al., 2021a; Lenoir et al., 2021c). Most commonly, a temporary

reduction in hepatic clearance and prolongation of half-life of elimination is observed. In some cases, the suspected interactions have led to elevated drug levels and drug-related adverse responses as observed for the CYP1A2 substrates, theophylline, and clozapine, the CYP2C19 and CYP3A4 substrate voriconazole and the CYP3A4 substrates tacrolimus and cyclosporine (Lenoir et al., 2021c). Similar interactions between infections or inflammation and hepatic cytochrome P450 metabolism are also observed in children but are less well studied overall compared to adults (Lenoir et al., 2021b).

Mechanisms of interactions. The regulation of hepatic cytochrome P450 metabolism by the innate immune response to infectious and inflammatory stimuli has been studied in preclinical animal models and human cellular models and involves a combination of transcriptional and post-translational mechanisms (Goralski et al., 2018; de Jong et al., 2020) as summarized in **Fig. 1** and detailed below. Briefly, pathogen-associated molecular patterns including microbial nucleic acids, lipopolysaccharides and other virulence factors serve as the primary stimuli and interact with membrane-bound or intracellular pattern recognition receptors (e.g. Toll-like receptors) on host tissue epithelium and tissue resident macrophages to stimulate the innate immune response and ensuing cytokine (e.g. interferons (IFN), interleukin (IL) -1β and IL-6 and tumor necrosis factor (TNF)) release (Medzhitov, 2010). In turn, cytokines, bind to their respective receptors on hepatocytes to activate nuclear factor kappa B (NF-κB) signaling, which directly represses transcription of some CYPs or reduces the nuclear localization or dimerization of transcription factors including pregnane X receptor, constitutive androstane receptor and aryl hydrocarbon receptor, resulting in reduced binding to CYP promoter regions and reduced CYP mRNA expression (de Jong et al., 2020; Stanke-Labesque et al., 2020). Direct binding of pathogen-

associated molecular patterns to toll-like receptors on hepatocytes, which activates NF-κB signaling, is an additional mechanism whereby CYPs can be downregulated. While not as well studied, epigenetic mechanisms and post-transcriptional regulation of CYP mRNA by microRNAs have also been demonstrated (Stanke-Labesque et al., 2020). In addition, the activation of inducible nitric oxide synthase 2 during the innate immune response increases nitric oxide which can regulate CYP enzyme function via transcriptional mechanisms, CYP degradation and catalytic inhibition (Donato et al., 1997; Morgan et al., 2020). The interactions between infectious stimuli are not limited to hepatic metabolism and clearance. Infectious and inflammatory stimuli have been reported to decrease intestinal and hepatic efflux transport, increase renal tubule drug transport, increase, or decrease blood-brain barrier drug efflux transport and decrease placental drug efflux transport (Goralski et al., 2018). Based on these broad interactions, there is potential for viral infections to impact drug absorption, drug distribution, drug metabolism and drug excretion processes.

SARs-CoV-2 infection and interactions with hepatic CYP metabolism. At the time of writing this article, an estimated 547 million people have been infected with SARS-CoV-2 (the virus causing COVID-19) and over 12,000,000,000 vaccine doses have been administered (Soriano et al., 2022). COVID-19 symptoms range from mild or asymptomatic in the least severe cases, to acute respiratory distress syndrome and death in the most severe cases (Garcia, 2020; Gao et al., 2021). Based on the understanding of interactions between the innate immune response and hepatic CYP metabolism, the downregulation of hepatic CYP is probable during SARS-CoV-2 due to increases in systemic concentrations of CYP-regulating cytokines, including IL-1β, IL-6, TNF and IFN-γ (Huang et al., 2020; Rodrigues et al., 2021). Of particular concern may be

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patients with severe COVID-19 who experience dysregulated pulmonary and systemic inflammatory responses with elevated IL-6 levels that correlate with disease severity and worse clinical outcomes (Chen et al., 2020; Wu et al., 2020; Lee et al., 2021; Yin et al., 2021). Clinical reports of patients with elevated levels of lopinavir, darunavir and clozapine during the pandemic provided initial evidence of downregulation of CYP3A4 and CYP1A2 in SARs-CoV-2-infected individuals (Cojutti et al., 2020; Cranshaw and Harikumar, 2020; Gregoire et al., 2020; Marzolini et al., 2020; Schoergenhofer et al., 2020). Adding strength to the initial evidence, a well-designed study that phenotyped SARS-CoV-2-infected patients during and after the infection using the Geneva cocktail approach found reduced CYP3A4, CYP2C19 and CYP1A2 metabolism, respectively, characterized by reduced metabolite to dose ratios of OH-midazolam/midazolam, OH-omeprazole/omeprazole and paraxanthine/caffeine (Lenoir et al., 2021d).

Historically, the impact of vaccines on hepatic CYP metabolism is less clear. As recently reviewed, most human studies or case reports have focused on influenza A vaccine interactions with the CYP2C9 substrate warfarin and CYP1A2 substrate theophylline with fewer studies of other CYPs or other vaccines (Lenoir et al., 2021c). Most studies did not find that the pharmacokinetics of drugs were significantly impacted; however, a few smaller studies or case reports suggested that interactions could occur in some individuals depending on patient factors (e.g. age, disease, basal CYP metabolism), drug and vaccine formulation and time after vaccination (Lenoir et al., 2021c). Theoretically, SARS-CoV-2 mRNA and adenoviral vaccine interactions with hepatic CYP metabolism are possible but there has been a lack of prospective studies in this regard.

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The ensuing symposium report begins with a discussion of the impact of SARS-CoV-2 infection and emergency use SARS-CoV-2 vaccines on hepatic CYP450 expression and function and explores the potential clinical implications of such interactions through discussion of a case report of transiently high clozapine levels in a patient treated with SARS-CoV-2 vaccine. Section three of the report discusses emerging evidence of inhibition of clearance of nanoparticles and biological therapies following SARS-CoV-2 infection and the potential risk for drug related toxicities. The report concludes with a discussion of the effects of viral infections on placental amino acid transport and their potenital to impact fetal development and a summary of unanswered questions and future research questions.

A case report exploring the possibility of SARS-CoV-2 vaccination increasing clozapine levels (*W.G.H.*)

Despite the challenges of prescribing and demands for monitoring, clozapine retains a unique place among antipsychotic drugs on account of proven efficacy in schizophrenia with limited response to other antipsychotic medications (Wagner et al., 2021). Side effects include metabolic disorders and weight gain, and effects on gastrointestinal, cardiovascular, pulmonary, neurological and hematological systems (Gurrera et al., 2022). Some side effects are associated with dose or plasma-level, others are more pronounced during dose escalation phases of treatment. Neurological, dose-related side effects of clozapine include sedation, abnormalities in the electroencephalogram, seizures, myoclonus, obsessive-compulsive symptoms, delirium; and through innervation of other organ systems: tachycardia, orthostatic hypotension, hypersalivation, and enuresis (Tio et al., 2021; Gurrera et al., 2022).

The risk of hematological side effects requires monitoring of white blood cell and absolute neutrophil counts during clozapine treatment. Following initiation of clozapine treatment, white blood cell counts (including neutrophils) typically rise slightly over the first week, fall to a slightly lower level than baseline over the second week, returning to baseline at the end of the second week (Blackman et al., 2021). During the first six weeks of treatment, levels of the cytokines tumor necrosis factor TNF and IL-6 rise, as does the level of the cytokine receptor soluble IL-2 (Roge et al., 2012). At steady state, the risk of neutropenia and rarely, agranulocytosis persists. The levels of immunoglobulins IgA, IgM and IgG are slightly low, as are the levels of class-switched memory B-lymphocytes (Ponsford et al., 2018; Ponsford et al., 2019).

Clozapine levels in blood are sensitive to activity of the main metabolizing enzyme, CYP1A2 (Eiermann et al., 1997). This activity is in turn sensitive to inhibition by drugs such as fluvoxamine, and to stimulation by exposure to smoked tobacco. Infection can increase clozapine levels in blood through an immunological cascade, beginning with antigen-presenting dendritic cells releasing cytokines detected by receptors in hepatocytes, leading to transcription factor expression and down-regulation of CYPs (Renton, 2005). An increase in the ratio of clozapine to the primary metabolite norclozapine suggests inhibition of CYP1A2 activity (Stanke-Labesque et al., 2020). During infection and inflammation, hepatocytes simultaneously release C-reactive protein, a biomarker indexing the inflammatory response (Sproston and Ashworth, 2018).

The COVID-19 pandemic created multiple challenges for clozapine treatment. The first was concern for continued access to hematological monitoring, resulting in a recommendation from

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an international working group for less frequent monitoring in well-stabilized patients (Siskind et al., 2020). An early retrospective cohort study raised concern that the usual hematological side effects of clozapine could increase risk for SARS-CoV-2 infection; this was not supported by a much larger study of antipsychotic-treated patients with severe infection (Govind et al., 2021; Ohlis et al., 2022). Clozapine-treated patients experiencing SARS-CoV-2 infection had a small decrease in neutrophils and lymphocytes over the first 7 days after becoming symptomatic, with return to normal levels by 14 days (Gee and Taylor, 2021). However, case reports indicated clozapine levels could rise as much as three-fold during SARS-CoV-2 infection, with an increase in the clozapine:norclozapine ratio (Tio et al., 2021). Dose reduction was recommended for clozapine-treated patients experiencing signs and symptoms consistent with clozapine toxicity (Siskind et al., 2020; Veerman et al., 2022).

Not surprisingly, the cellular and molecular response to vaccination for SARS-CoV-2 shares features of infection (Sahin et al., 2020). Neutrophils increase slightly and lymphocytes decline after 2 days, returning to normal after 8 days. Cytokine release occurs, and C-reactive protein rises transiently at day 2. These observations during the registration studies of an mRNA vaccine suggested that similar effects in clozapine-treated patients could be associated with increased clozapine level, and possible toxicity. We reported a case with findings suggestive of such an effect (Thompson et al., 2021).

In brief, a 51-year-old nonsmoking man with a long history of schizoaffective disorder had been treated with clozapine for over 10 years. He received an mRNA vaccine (Pfizer-BioNTech) for SARS-CoV-2, and 4 days later developed delirium, falls, incontinence and tachycardia (pulse rate 129/min). He was hospitalized due to concern for infection as the cause of these symptoms –

however, repeated SARS-CoV-2 PCR tests were negative, as were other tests for infection. **Fig. 2** provides a graphical representation of risk factors, clinical and laboratory findings, and course of illness in four domains: a) diagnosis of the presenting problem, b) patient-related factors, c) co-morbidities, and d) treatment-related (vaccine and clozapine) (Thompson et al., 2021). Past experience included a non-COVID pneumonia 3 months previously, with elevation of neutrophils and slight lowering of lymphocytes, associated with a high C-reactive protein level, as expected with an acute infection. As well, the clozapine level and clozapine:norclozapine ratio were elevated. Of note, following the resolution of pneumonia, the clozapine:norclozapine ratio remained > 2.0, suggesting the patient was a slow metabolizer of clozapine at baseline. Multiple comorbidities were noted, with sleep apnea possibly increasing risk for neurological toxicity of clozapine. Elevation of clozapine level, and clozapine:norclozapine ratio was detected on admission, as well as a high C-reactive protein level and neutrophil/lymphocyte responses similar to the previous pneumonia, all consistent with the pathway from immune stimulation to CYP inhibition described above. Interestingly, a computed tomography scan revealed an unsuspected normal pressure hydrocephalus, a neurological finding that could also contribute to the risk for delirium and other symptoms associated with an acute increase in clozapine level. Clozapine was held for two days, the level fell from 1078 ng/mL (3296 nM/L) to 396 ng/mL (1212 nM/L), and the clozapine/norclozapine ratio declined from 3.75 to 1.57. The symptoms of toxicity cleared.

This single case study provides a temporal sequence of associations that support transient clozapine toxicity as the origin of symptoms. Adopting the approach to clinical and laboratory findings illustrated in **Fig. 2** is analogous to an organizational strategy used to report the clinical expression of pathophysiology in HIV-related neuropsychiatric disorders, and may have wider value (Ances and Letendre, 2019). Observations from case studies need to be supported or

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discounted based on findings in larger cohort studies. A report of the effects of SARS-CoV-2 vaccines in 139 patients treated with clozapine found no clinically significant changes in symptoms or hematological parameters, but did support the observation from the present case of an increase in median clozapine level (after second dose) of effect size 0.28 and p=0.003 (Veerman et al., 2022). Larger studies such as this could provide the opportunity to investigate potentially complex interactions that could occur with CYP1A2 activity depending on concurrent exposures, such as the smoking status of patients. To summarize, case-based and cohort studies conclude that SARS-CoV-2 vaccination is indicated and safe in patients treated with clozapine, and supplementing with reminders on watching for symptoms or signs of clozapine toxicity after vaccination may be a useful precautionary measure to help maintain patients safely on clozapine therapy.

Impact of emerging infections and vaccines on CYP450 metabolism (*M.A.C.*)

People with highest risk of complications from COVID-19 are those with underlying medical conditions (cardiovascular disease, diabetes, hypertension, chronic lung disease) which require use of several medicinal agents for maintenance therapy (Peng et al., 2021; Touyz et al., 2021; Conway et al., 2022; Geca et al., 2022). Although there are few studies in the literature that specifically focus on how virus infection imapcts hepatic and renal drug metabolism, there are data to suggest that drug metabolism will be impacted during various stages of SARS-CoV-2 infection and after administration of emergency use vaccines.

Active Infection. The most obvious time of concern is during active infection with SARS-CoV-2, virus production and shedding starts several days before the inflammatory phase where

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circulating cytokines can suppress hepatic and renal drug metabolism (**Fig. 3**). However, changes

in CYP expression and function could start as soon as SARS-CoV-2 enters the kidney and liver

tissues as the spike protein has been shown to engage integrins as well as angiotensin-converting

enzyme 2 receptors for cell entry (**Fig. 4A**,(Liu et al., 2022)). Integrins are heterodimeric

receptors, consiting of an α and β subunit (**Fig. 4B**). Activation of the integrin receptors is tightly

regulated and bidirectional and is mediated through binding of external stimulators (virus,

bacteria, RGD rich peptides) or internally through the binding of the intracellular protein talin

with the β subunit of the receptor (Kadry and Calderwood, 2020). Talin-mediated signalling is

essential for productive SARS-CoV-2 infection and evidence suggests it serves as a molecular

link between infection and CYP3A function (Simons et al., 2021). In support of this, the removal

of RGD integrin binding domains from the capsid of a recombinant adenovirus and treatment of

human hepatocytes with small interfering RNA specific for the β subunit of integrin receptors

reversed the suppression of CYP3A activty and expression normally seen during infection in

mice (Jonsson-Schmunk et al., 2016). Additionally, new data from our laboratory show that

silencing of talin in uninfected human hepatocytes increases CYP3A4 metabolic capacity 2.5-

fold (**Fig. 4C**). Taken togther this suggests that the ability of drugs to be cleared by the kidney or

the liver is compromised at the time the virus spike protein engages integrin receptors (**Fig. 4A**).

Cytokine production in response to the virus further establishes this effect (**Fig. 3**). While hepatic

and renal CYPs have been shown to return to baseline levels once virus infection has resolved

(Callahan et al., 2005; Le et al., 2006; Croyle, 2009), the suppression of CYPs may be extended

in the case of severe and post-acute COVID-19 syndrome, both characterized by hyper- and

extended inflammatory states (**Fig. 3**, (Nalbandian et al., 2021; Lamers and Haagmans, 2022)).

Post-Acute COVID-19 Syndrome (PACS)/Re-Infection. The American Centers for Disease Control and Prevention defines PACS as a variety of new, returning, or ongoing health problems that people experience 4 or more weeks after first being infected with SARS-CoV-2 while the World Health Organization defines it as a condition that occurs 3 months from the onset of symptomatic COVID-19 that lasts for at least 2 months and cannot be explained through alternative diagnosis (Chippa et al., 2022; Soriano et al., 2022). PACS is associated with presistant virus, viral antigen and viral RNA in tissues such as the liver and kidney that create chronic inflammation as well as autoimmune states (Ramakrishnan et al., 2021; Copur et al., 2022). This provides the conditions that could lead to a prolonged significant impairment of CYP expression and activity in patients with PACS. It is also important to note that post-acute syndromes also occur with other viral pathogens such as West Nile and influenza viruses (Choutka et al., 2022). To date, the impact of PACS on renal and hepatic drug metabolism has not been evaluated in animal models of disease nor the clinic. As the pandemic progressed, reports of re-infection with different SARS-CoV-2 variants and breakthrough infections in vaccinated pateints occurred at an increasing rate (Negi et al., 2021; Mohseni Afshar et al., 2022). Although this phenomenon is common with other viruses such as influenza, the impact of re-infection on hepatic and renal drug metabolism has not yet been evaluated.

Immunization. To date, over 12 billion people have recived at least one dose of a COVID-19 vaccine with approximately 5 billion successfully receiving a complete initial protocol (Mathieu et al., 2021). The most commonly observed adverse reactions to these vaccines include erythema, fever, fatigue, headache and hypersensitivity reactions (Kouhpayeh and Ansari, 2022; Lau and Vadlamudi, 2022). These types of reactions are transient and usually resolve within 48-72 hours. They are also commonly associated with other vaccines and are caused by the production and

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release of cytokines into the circulation (2011; Herve et al., 2019). While several reports have

described interactions between drugs with narrow therapeutic windows and influenza vaccines

(Raaska et al., 2001; Robertson, 2002; Carroll and Carroll, 2009), these results have not been able

to be fully replicated in controlled clinical trials (Stults and Hashisaki, 1983; Gomolin et al.,

1985; Soontornpun et al., 2020) which suggests that patient specific characteristics such as

altered drug clearance prior to immunization may also be required for notable observation of

changes in CYP soon after immunization (Meredith et al., 1985). To date, the impact of boosting

doses of a vaccine has not been investigated in the clinic nor in animal models of drug

metabolism.

Different COVID-19 vaccines may alter CYP enzymes in different ways. Studies in which the

Ebola Zaire glycoprotein was expressed from a recombinant adenovirus vaccine (similar in

approach to the Janssen and Astra Zeneca COVID-19 vaccines) found that hepatic CYP enzymes

were mildly suppressed in mice 24 hours after immunization and resolved by 48 hours (Jonsson-

Schmunk et al., 2021). Treatment with glycoprotein alone at concentrations found in the

circulation of immunized individuals significantly induced CYP3A4 in human hepatocytes while

treatment with a H1N1 human influenza virus almost completely shut down CYP expression and

activity. Influenza held such a profound effect on CYP through its ability to control RNA

translation capacity within the cell during active infection. This, paired with the fact that specific

components of the lipid nanoparticles in which the Moderna and Pfizer vaccines were formulated

triggered a broad inflammatory response (Jonsson-Schmunk et al., 2021; Kenigsberg et al., 2022;

Padin-Gonzalez et al., 2022; Szebeni et al., 2022; Tahtinen et al., 2022) suggests that these

vaccines have the potenital to significantly impact hepatic and renal CYP mediated drug

metabolism. This is just starting to be reported in the clinic as demonstrated through the

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clozapine case report in the previous section. Further study of the impact of SARS-CoV-2 infection and immunization are currently underway in relevant animal models of disease that also correlate with human CYP expression and activity patterns (Tiwari et al., 2022).

Role of the Innate Immune System in COVID-19 Infections and the Dispositon of Complex Drugs (*W.C.Z and M.H.*).

The innate immune system is involved in the recognition, uptake and clearance of a wide range of pathogens and substances, such as bacteria, viruses, nanoparticles, and biologics (Lucas et al., 2015; Madden et al., 2017; Lucas et al., 2018). Antigen presenting cells of the innate immune system are the first line of contact and mediators of the immune response against the viruses, such as the coronavirus (**Fig. 5**) (Arancibia et al., 2007; Takeuchi and Akira, 2009). There is high variability in the infection rate, severity and response to treatment of COVID-19 (Kakodkar et al., 2020; Zimmer, 2020; Rahman et al., 2021). The most severe cases of COVID-19 appear to include an over-reactive or hyper-inflamed immune response in the lungs that lead to severe pneumonitis and death.

Drug metabolism and disposition can be altered by the innate immune system, which is the primary pharmacokinetic clearance pathway for complex drugs (e.g. nanoparticles, conjugates, and biologics) (Lucas et al., 2015; Lucas et al., 2018). Patients with SARS-CoV-2 infections may have altered innate immune system function and phenotypes, which alters the recognition, uptake and clearance of nanoparticles and biologics putting them at higher risk for sub-optimal response or drug related toxicities. This section describes factors that alter the function of the innate

immune system, the interaction between the innate immune system and complex drugs, and the effects of SARS-CoV-2 on the innate immune system and alteration of the pharmacokinetics of complex drugs, such as Pegylated (PEG)-liposomal doxorubicin (Doxil[®]) and monoclonal antibodies and antibody drug conjugates (ADCs).

Biomarkers of Innate Immune System (innate immune system). There is a high (~10-fold) inter-patient variability in the phenotype of function (e.g., phagocytosis, reactive oxygen species generation) and surface receptors (e.g., FcɣRs) of innate immune system cells in blood and tissues (Lucas et al., 2015; Lucas et al., 2018). This variability in the innate immune system biomarkers is associated with high variability in immune response and pharmacology of nanoparticles, viral drug carriers and antibodies that are identified and taken up by the innate immune system cells (Lucas et al., 2015; Madden et al., 2017; Lucas et al., 2018). Moreover, the increased severity and death rate of COVID-19 in male patients and overweight patients are consistent with our results of higher innate immune system function as measured by the biomarkers in these patient groups (Zamboni et al., 2009; La-Beck et al., 2012; Klang et al., 2020; Papadopoulos et al., 2021). Thus, we hypothesized that interactions between the SARs-CoV-2 virus and the innate immune system will alter the function of the innate immune system and change the pharmacokinetics and pharmacodynamics of complex drugs, such as PEG-liposomal doxorubicin and antibodies/ADCs.

Effect of SARs-CoV-2 infection on innate immune system Function and pharmacokinetics of PEG-liposomal doxorubicin in Mice. Viral load and biomarkers of the innate immune system were evaluated on day 2 and 4 in MA-10 mouse adapted SAR-CoV-2 in wild type BALB/c mice (n=4/time point) infected with and without SARS-CoV-2 virus (Leist et al., 2020).

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Viral load was measured by quantitative reverse transcriptase polymerase chain reaction (Geng et al., 2021). Biomarkers of innate immune system function in blood (phagocytosis of monocytes) was evaluated using previously validated and published methods (Caron et al., 2013; Lucas et al., 2017). Previously published models describing the relationship between innate immune system function (phagocytosis of monocytes in blood) and the disposition of PEG-liposomal doxorubicin in plasma was used to simulate the plasma pharmacokinetic disposition of PEG-liposomal doxorubicin in mice with and without SARS-CoV-2 virus infection.

The SARS-CoV-2 virus load on days 2 and 4 after infection were $1,493,750 \pm 379,487$ and $209,000 \pm 321,775$ plaque forming units, respectively. Phagocytic function was similar in infected and non-infected mice on day 2 post infection ($p > 0.05$). However, on day 4 post infection the phagocytic function was 2.3-fold higher in the infected mice ($67,425 \pm 18,379$ mean fluorescence intensity, MFI) compared to non-infected mice ($29,283 \pm 4,338$ MFI) ($p < 0.001$). Interestingly on day 4 post infection, there appears to be a slight inverse relationship between viral load and phagocytic function in monocytes in blood (**Fig. 6**). Thus, the amount of viral load may also affect the degree of change in innate immune system function and change in pharmacokinetics and pharmacodynamics of complex drugs. Based on our prior studies measuring and modeling the relationship between phagocytic function of monocytes in blood and the pharmacokinetics of PEG-liposomal doxorubicin in plasma of mice and humans, pharmacokinetic simulations predict that the 2.3-fold higher phagocytic function in infected mice will increase PEG-liposomal doxorubicin clearance by ~2-fold and decrease the plasma exposure of PEG-liposomal doxorubicin by at least half compared to non-infected mice (**Fig. 7**). These results strongly suggest that COVID-19 infection alters the function of the innate immune system, which would then alter the pharmacokinetics and pharmacodynamics of complex drugs,

such as PEG-liposomal doxorubicin. Thus, the doses of complex drugs designed for non-infected patients may not be optimal for patients with COVID-19.

Impact and Future Directions. The ultimate goal of this research is to translate the biomarkers of the innate immune system evaluated in this study into novel clinical tests that predict variability and changes in innate immune system function in patients with and without COVID-19 and other types of infections. These results can then be used to optimize the dose of complex drugs in these patient populations. Ongoing studies are evaluating the pharmacokinetic dispositon of PEG-liposomal doxorubicin in plasma and tissue (e.g., liver, spleen, and lung, which are the primary organs of the innate immune system and depot sites for complex drugs, such as PEG-liposomal doxorubicin) in mice without and with infection with SARS-CoV-2 virus to validate the simulated plasma pharmacokinetic results and to evaluate the disposition PEG-liposomal doxorubicin in tissues. In addition, other biomarkers of PEG-liposomal doxorubicin and innate immune system function and phenotype in blood, liver, spleen, and lungs are also being evaluated for other complex drugs including antibodies and ADCs.

It is important to understand the acute (e.g., a few days post infection), late (e.g., weeks post infection), and prolonged (e.g., months to years) effects of SARS-CoV-2 infection on the function of the innate immune system and alteration in the pharmacokinetics and pharmacodynamics of complex drugs (Healey et al., 2022). It is also important to understand these effects in asymptomatic SARS-CoV-2 infected patients who may be more likely to undergo treatment for their underlying disease (Gao et al., 2021). As the COVID-19 vaccines by Moderna and Pfizer/BioNTech are nanoparticle or carrier agents, it would be important to understand how these and other nanoparticle based vaccines alter the innate immune system and potentially the

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pharmacokinetic dispositon, efficacy, and toxicity of complex drugs in the general population,

but especially in high risk populations, such as obese or immune suppressed patients, that may

already have an altered innate immune system (Meo et al., 2021).

Infection during pregnancy downregulates the expression of amino acid transporters in rat and human placentas *(E.R.M and M.P-M.)*

Infection with SARS-CoV-2 and resulting COVID-19 carries an increased risk during pregnancy.

Pregnant women who are infected with SARS-CoV-2 have an increased risk of severe COVID-

19, including increased risk of hospital admission, intensive care unit admission, and requiring

mechanical ventilation (Safadi et al., 2022). Moreover, despite the fact that risk of vertical

transmission of SARS-CoV-2 is estimated to be under 5%, maternal infection during pregnancy

also increases the risk of negative fetal outcomes including preterm birth, decreased birth weight,

fetal distress in labour, neonatal intensive care unit admission, and stillbirth (Giuliani et al., 2022;

Safadi et al., 2022). Additionally, it is possible that prenatal SARS-CoV-2 infection may have

long-term implications for offspring neurodevelopment. It is well established that various

bacterial and viral infections, when contracted during pregnancy, increase the chance of the

offspring having a neurodevelopmental disorder such as autism spectrum disorder or

schizophrenia (Sorensen et al., 2009; Atladttir et al., 2010; Brown and Derkits, 2010; Jiang et al.,

2016; Giuliani et al., 2022). Emerging evidence suggests that offspring resulting from

pregnancies complicated by COVID-19 may exhibit developmental delays, implying that SARS-

CoV-2 infection may similarly increase the chance of neurodevelopmental disorders (Edlow et

al., 2022). However, as with the other infections linked to changes in offspring

neurodevelopment, how infection during pregnancy alters fetal brain development is unclear.

The gold standard model for investigating the link between viral infection during pregnancy and neurodevelopmental changes involves rodents receiving prenatal administration of the synthetic, double-stranded RNA molecule polyinosinic:polycytidylic acid (poly(I:C)). Poly(I:C) stimulates toll-like receptor 3, the same receptor stimulated by many viruses, including SARS-CoV-2 (Bortolotti et al., 2021). While SARS-CoV-2 also stimulates toll-like receptor 7 (which senses single strand RNA), an animal model of toll-like receptor 7 agonism during pregnancy has yet to be extensively characterized. Administration of poly(I:C) during pregnancy results in altered behaviour and neurobiology in rodent offspring that are consistent with neurodevelopmental disorders such as autism spectrum disorder and schizophrenia (Careaga et al., 2017; Haddad et al., 2020). Using the poly(I:C) rodent model, studies have concluded that the link between prenatal infection and neurodevelopmental disorders is due to activation of the maternal immune system itself, termed "maternal immune activation", as opposed to the presence of a particular pathogen. However, how maternal immune activation alters fetal brain development remains unknown.

A potential link between maternal immune activation and neurodevelopmental changes may lie in changes to transport of essential nutrients across the placenta. During pregnancy, the placenta supports fetal development by acting as a protective barrier between maternal and fetal circulation and facilitating exchange of nutrients and waste. To support both functions, the placenta expresses a number of ATP-binding cassette and solute carrier drug and nutrient transporters (Lager and Powell, 2012; Dallmann et al., 2019). Inflammation and infection has been shown to alter expression of drug transporters in the placenta (Petrovic and Piquette-Miller, 2010; Evers et al., 2018), resulting in altered fetal drug exposure (Petrovic and Piquette-Miller,

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2015). In contrast, the impact of infection or inflammation on placental nutrient transporters is less well characterized.

Amino acid transport across the placenta is best conceived as a systems approach, in which the concerted effort of accumulative transporters, exchangers, and facilitated transporters with overlapping substrate specificity drive a net transport of amino acids from maternal to fetal circulation (Cleal et al., 2018). Amino acid transporters are also expressed in the fetal brain at the blood-brain barrier, where they facilitate amino acid transport into and out of the fetal brain (Campos-Bedolla et al., 2014), as well as at neuronal synapses, where they help regulate neurotransmission (Nguyen et al., 2022). Regulation of amino acid transport across the placenta and throughout the fetal brain is essential, as amino acids are required for fetal brain development (McDonald and Johnston, 1990; Kurbat and Lelevich, 2009; Tabatabaie et al., 2010; Tochitani, 2017). As such, changes in placental or fetal brain amino acid transport could alter fetal access to the amino acids required for neurodevelopment and contribute to a neurodevelopmental disorder. Indeed, amino acid dysregulation has been implicated in both autism spectrum disorder and schizophrenia (Bala et al., 2016; Saleem et al., 2017).

We therefore hypothesized that maternal immune activation associated with prenatal viral infections could alter placental and fetal brain amino acid transporter expression, which could alter brain development. To test this hypothesis, we used the rat poly(I:C) model of maternal immune activation which is known to cause neurodevelopmental changes in offspring (Haddad et al., 2020). At 24-48 hours after poly(I:C) administration, significant decreases in the expression of alanine serine cysteine transporter 1 (ASCT1) and excitatory amino acid transporter 2 (EAAT2) were found in the placenta, with a similar trend for the small neutral amino acid

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transporter 2 (SNAT2) (McColl and Piquette-Miller, 2019) (**Table 1**). Poly(I:C) also imposed

significant decreases in the expression of SNAT5, EAAT1, and glycine transporter 1 (GLYT1) in

fetal brain. Interestingly, inherited deficiencies in ASCT1, EAAT1 or 2, and GLYT1 are

associated with neurological changes in humans (Yahyaoui and Prez-Fras, 2020). HPLC analysis

of the fetal brains also revealed significant changes in tissue concentrations of multiple amino

acids (McColl and Piquette-Miller, 2019). Of note, poly(I:C)-mediated maternal immune

activation altered the levels of several amino acids required for proper brain development. This

included a significant decrease in taurine, which is necessary for optimal neuronal proliferation

(Tochitani, 2017), as well as significant decreases in glycine and gaba aminobutyric acid

(GABA), two neurotransmitters that help regulate fetal brain development (Tochitani, 2017).

Moreover, some of the affected amino acids have been implicated in neurodevelopmental

disorders. For example, decreased serum taurine levels have been proposed as a biomarker for

autism spectrum disorder (Park et al., 2017) and altered GABAergic function has been implicated

in both autism spectrum disorder and schizophrenia (Lewis et al., 2005; Cetin et al., 2015).

Therefore, placental, and fetal brain amino acid transport are altered in a rat model of viral

infection that is associated with changes in offspring neurodevelopment.

We next sought to determine whether similar changes occur in human pregnancies complicated

by infection. To do so, we obtained human placentas collected at term from pregnancies

complicated by chorioamnionitis or suspected active infection of unknown origin, and gestational

age-matched controls. Chorioamnionitis is characterized by inflammation of pregnancy tissues,

including the placenta, typically as a result of an ascending bacterial infection (Cappelletti et al.,

2020). Placentas in the suspected infection group were from individuals who had not had an

official diagnosis of infection, but were experiencing symptoms such as fever, chills, or elevated

white blood cell count when they delivered. In line with what we saw in poly(I:C)-treated rats, suspected infection was associated with a significant decrease in membrane protein expression of ASCT1, and a trend towards 50% downregulation of SNAT2 in human placentas (McColl and Piquette-Miller, 2022) (**Table 1**). In contrast, ASCT1, EAAT2, and SNAT2 expression were unchanged in chorioamnionitis placentas. As many of these individuals were prescribed antibiotics earlier in pregnancy, it is likely that the infection and inflammation were resolved before their placentas were collected at term.

Overall, our results demonstrate that a viral model of maternal immune activation in rats which is known to alter offspring neurodevelopment also exhibits altered placental and fetal brain amino acid transport (McColl and Piquette-Miller, 2019). These changes are also partially recapitulated in placentas from humans experiencing an active infection (McColl and Piquette-Miller, 2022). Given the importance of amino acids during fetal brain development and their implications in disorders such as autism spectrum disorder and schizophrenia, these changes could contribute to the link between viral infection during pregnancy and offspring neurodevelopmental disorders. Thus, our results demonstrate that subsequent research needs to not only consider how SARS-CoV-2 infection may impact the transport or metabolism of xenobiotics, but also endogenous nutrients, particularly in the context of infection during pregnancy. Whether prenatal SARS-CoV-2 infection will be associated with increases in offspring neurodevelopmental disorders remains to be seen, but long-term follow-up studies tracking neurodevelopment after prenatal COVID-19 are taking place.

Discussion

Emerging research indicates that interactions between SARS-CoV-2 infection or vaccination on hepatic CYP metabolism is possible and can lead to reduced metabolism of small molecule drugs. Furthermore, through effects on the innate immune system, alteration in the metabolism of complex drugs (e.g. liposomal formulations) are also possible. Despite these advances, there remain several gaps in understanding that should be addressed with future research. There is a need to better understand in which situations and for which drugs these interactions could impact drug safety and effectiveness and if modifications in drug dosing or therapeutic monitoring are required. Additional studies to evaluate the interactions between SARs-CoV-2 and other aspects of drug disposition such as effects on blood-brain barrier drug transporters and renal tubule transporters, and the respective impact on central nervous system drug distribution and renal drug elimination are required. Research is needed to determine if altered drug disposition persists in individuals with long COVID and if this contributes to observed symptoms associated with long COVID. Additional research on novel mRNA and adenoviral vaccine technologies including booster doses and impacts on the disposition of other drug therapies is also required. Until these questions are answered it is important to remain aware that SARS-CoV-2 infection mediated interactions with the hepatic CYP metabolism are possible and a potential source of variability in drug responses.

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Authorship Contributions

Participated in research design (Eliza R. McColl, Maria A. Croyle, William C. Zamboni, Mark Heise and Micheline Piquette-Miller)

Conducted experiments (Eliza R. McColl, Maria A. Croyle, William C. Zamboni and Mark Heise)

Performed data analysis (Eliza R. McColl, Maria A. Croyle, William G. Honer, William C. Zamboni and Mark Heise)

Wrote or contributed to writing the manuscript (Eliza R. McColl, Maria A. Croyle, William G. Honer, William C. Zamboni, Micheline Piquette-Miller and Kerry B. Goralski)

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Footnotes

Conflicts of Interest: William C. Zamboni is an inventor of the biomarkers of the innate immune system and founder and co-owner of Glolytics, LLC company that is developing these biomarkers. Eliza R. McColl, Maria A. Croyle, William G. Honer, Mark Heise, Micheline Piquette-Miller and Kerry B. Goralski have no conflicts to report.

Legends for Figures

Figure 1: General overview of hepatic CYP regulation by infection and inflammation.

Figure abbreviations, nitric Oxide (NO), nitric oxide synthase (NOS), Cytochrome P450 (CYP),

IL (Interleukin), Interferon (INF) and Tumor necrosis factor (TNF).

Figure. 2. Aggregate illustration of clinical and laboratory findings in a case of delirium

developing four days after SARS-CoV-2 vaccination. Laboratory findings relevant to the

diagnostic process are illustrated in graphs, the computed tomography scan shows evidence of an

unsuspected structural brain abnormality, normal pressure hydrocephalus. Figure abbreviations:

neutrophils (PMN), lymphocytes (lym), 3 months prior to vaccination baseline (-3mon),

gastroesophageal reflux disease (GERD) and pre-vaccination (pre-vacc).

Figure 3: Time Course of SARS-CoV-2 Infection with Respect to Severity of Disease: Virus

Shedding, Inflammatory Phase, Symptom Development and Therapies. Note the

inflammatory phase lasts approximately 15 days in mild cases and is more amplified and

extensive in severe cases suggesting that CYP mediated metabolism could be altered for

extensive periods of time even with mild infections. **Image source:** ViralZone: a knowledge

resource to understand virus diversity, Viral Zone, SIB Swiss Institute of Bioinformatics (Hulo et

al., 2011). URL https://viralzone.expasy.org/9116 (link accessed June 26, 2022).

Figure 4. Engagement of Integrins by the SARS-CoV-2 Spike Protein Could Induce

Changes in Hepatic CYP expression and Function. A. SARS-CoV-2 spike protein facilitates

virus entry through engagement of beta integrins and angiotensin converting enzyme 2 (ACE2)

receptors. **B**. Engagement of integrins through the spike protein stimulates outside in cell

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signaling pathways that can suppress CYP expression and function. Talin can engage integrins from the cytoplasm and alter CYP through inside out signaling pathways. **C**. Suppression of Talin in human hepatocytes increases CYP activity suggesting that this protein, which supports SARS-CoV-2 infection, plays a notable role in the regulation of CYP.

Figure 5: The Innate immune system is the first line of defense against pathogens, such as viruses. The innate immune system response activates adaptive immune processes through Toll-like receptors (TLRs) and antigen presentation. Innate immune cells recognize and phagocytose pathogens that promote cytokine & antigen presentation to T cells. In addition, these same innate immune cells are directly involved in the identification, uptake, and clearance of complex drugs (e.g., nanoparticles, conjugates, and biologics).

Figure 6: The relationship between viral load measured by plaque-forming units (PFU) and phagocytic function (mean fluorescence intensity, MFI) of monocytes in blood of infected mice on day 4 post infection. There is a slight inversion relationship between viral load and phagocytic function. Thus, the amount of viral load may also affect the degree of change in innate immune system function and change in pharmacokinetic and pharmacodynamic disposition of complex drugs.

Figure 7: Simulated plasma concentration versus time curves of liposomal encapsulated doxorubicin in mice. Non-infected mice administered a single intravenous dose of PEG-liposomal doxorubicin (6 mg/kg) are shown by the black line. Infected mice administered a single IV dose of PEG-liposomal doxorubicin (6 mg/kg) are shown in red. Infected mice

administered a single intravenous dose of PEG-liposomal doxorubicin (12 mg/kg) are shown in

green.

Table 1: Summary of changes in amino acid transporter membrane protein expression in the placenta and fetal brain after poly(I:C) administration to rats (McColl and Piquette-Miller, 2019) or suspected active infection in humans (McColl and Piquette-Miller, 2022).

Transporter	Gene Name	Substrate(s)	Localization	Mechanism	Impact of Poly(I:C) (Rats)	Impact of Suspected Infection (Humans)
Placenta (Cleal et al., 2018)						
ASCT1 (Alanine serine cysteine transporter 1)	*SLC1A5*	Alanine, serine, cysteine, threonine	Basal membrane	Na^+-dependent exchanger	↓75%*	↓75%*
EAAT2 (Excitatory amino acid transporter 2)	*SLC1A2*	Glutamate, aspartate	Microvillous membrane and basal membrane	Na^+/H^+/amino acid cotransport/K^+ exchange	↓50%*	↔
SNAT2 (Small neutral amino acid transporter 2)	*SLC38A2*	Glycine, proline, alanine, serine, cysteine, glutamine, asparagine, histidine, methionine	Microvillous membrane	Na^+/amino acid cotransport, H^+ antiport	↓20% (trend)	↓50% (trend)
Fetal Brain (Harvey and Yee, 2013; Brer, 2014; Zaragoz, 2020)						
EAAT1 (Excitatory amino acid transporter 1)	*SLC1A3*	Glutamate, aspartate	Abluminal side of BBB, astrocytes, neurons	Na^+/H^+/amino acid cotransport/K^+ exchange	↓40%*	
GLYT1 (Glycine transporter 1)	*SLC6A9*	Glycine	Astrocytes and neurons	N^+/amino acid cotransport	↓40%*	
SNAT5 (Small neutral amino acid transporter 5)	*SLC38A5*	Glycine, glutamine, alanine, serine, Cysteine histidine, asparagine	Astrocytes	Na^+/amino acid cotransport, H^+ antiport	↓25%*	

↓ signifies decreased expression, ↔ signifies no change in expression. * significantly different from controls, $p<0.05$
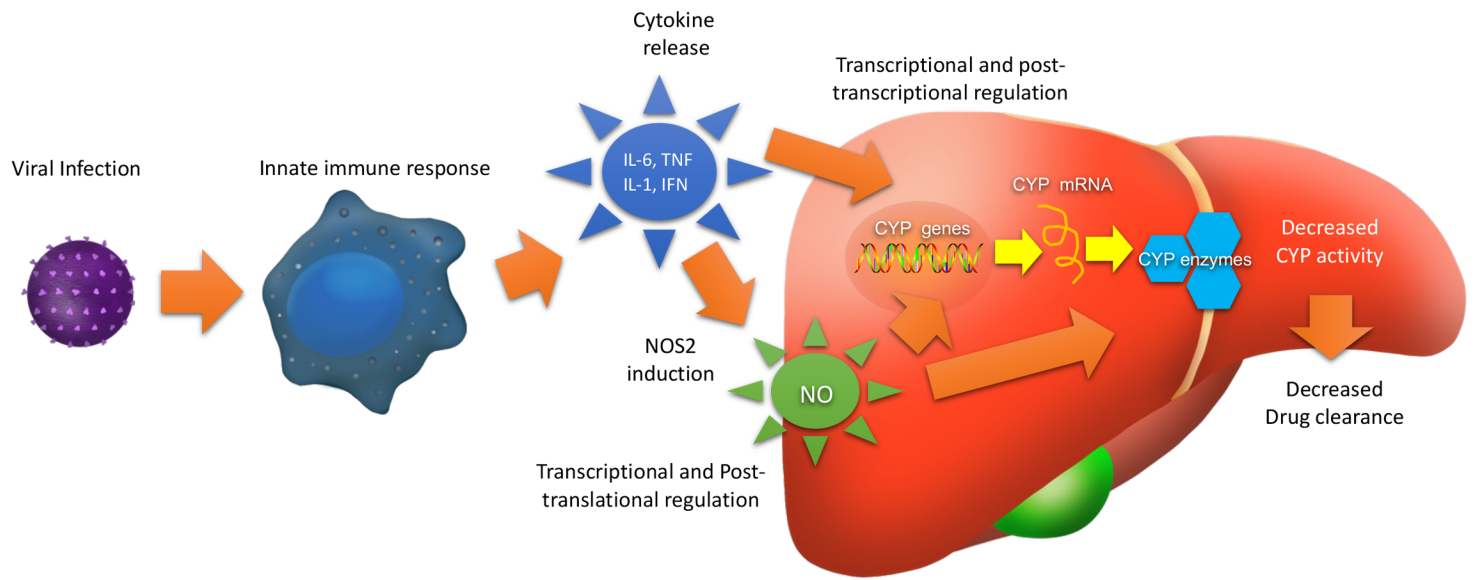
Figure 1

Figure 2

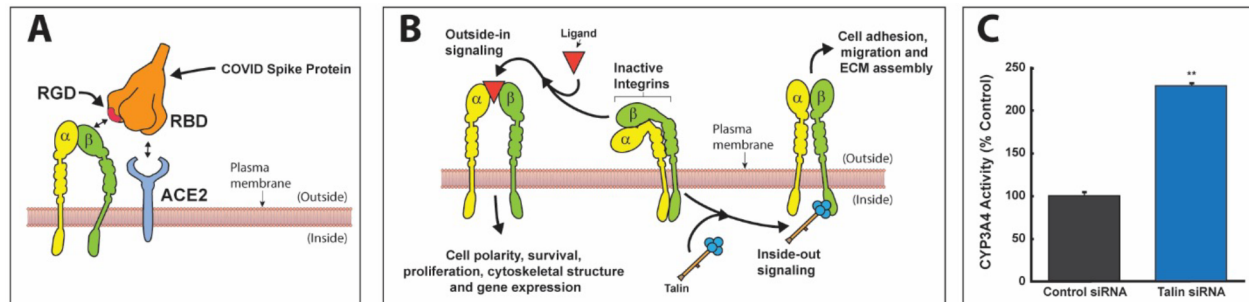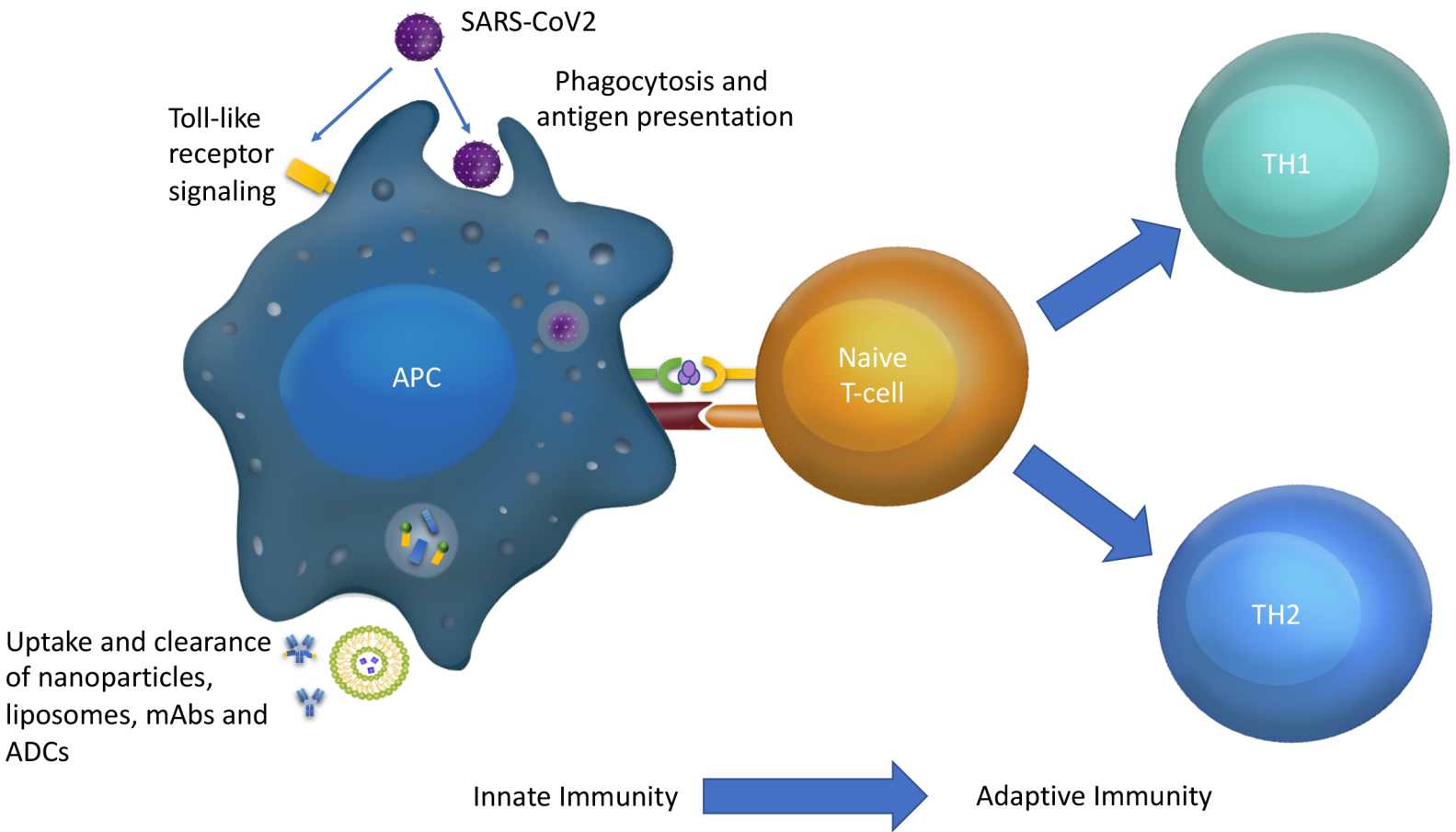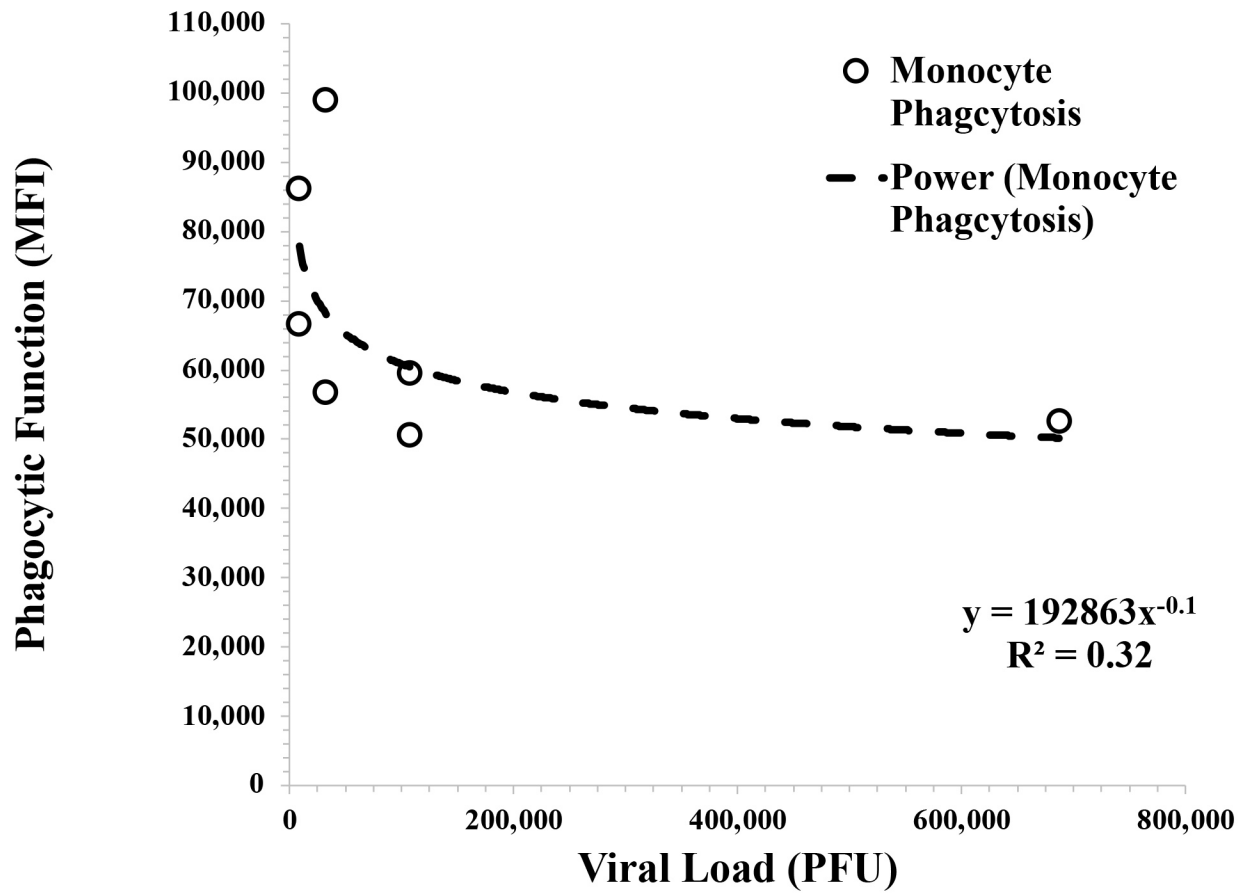| Time	Past experience	Baseline	4-6 days after vaccine
Diagnosis of presenting problem	Pneumonia		Delirium
Patient-related factors			
Co-morbidities	Schizoaffective disorder	1. Schizoaffective 2. Type-II diabetes 3. GERD 4. Hyperlipidemia 5. Obesity 6. Obstructive sleep apnea	
Treatment-related factors	Clozapine	1. SARS-CoV-2 vaccination first dose 2. Clozapine continued	

Figure 3
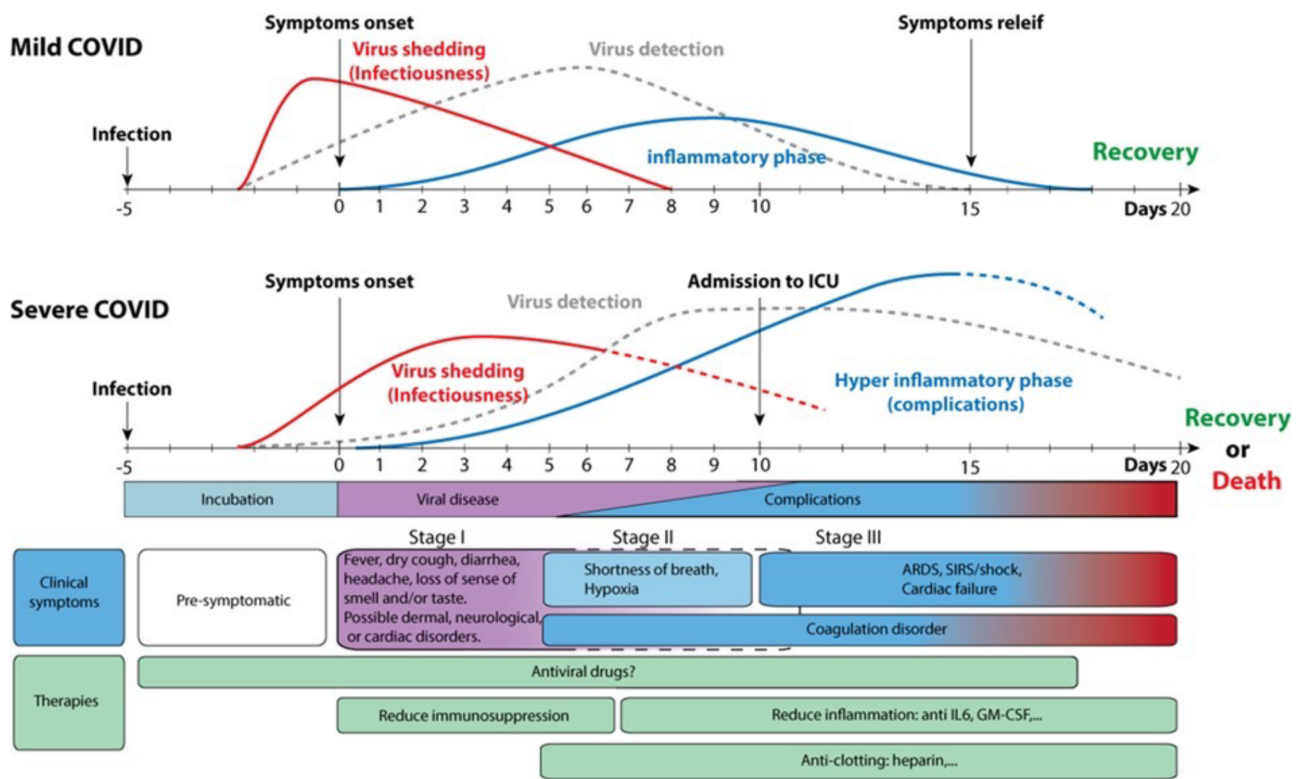
Figure 4

Figure 5

Figure 6

Figure 7