



## Review

# Impact of immune enhancement on Covid-19 polyclonal hyperimmune globulin therapy and vaccine development



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## ABSTRACT

The pandemic spread of a novel coronavirus – SARS coronavirus-2 (SARS-CoV-2) as a cause of acute respiratory illness, named Covid-19, is placing the healthcare systems of many countries under unprecedented stress. Global economies are also spiraling towards a recession in fear of this new life-threatening disease. Vaccines that prevent SARS-CoV-2 infection and therapeutics that reduces the risk of severe Covid-19 are thus urgently needed. A rapid method to derive antiviral treatment for Covid-19 is the use of convalescent plasma derived hyperimmune globulin. However, both hyperimmune globulin and vaccine development face a common hurdle – the risk of antibody-mediated disease enhancement. The goal of this review is to examine the body of evidence supporting the hypothesis of immune enhancement that could be pertinent to Covid-19. We also discuss how this risk could be mitigated so that both hyperimmune globulin and vaccines could be rapidly translated to overcome the current global health crisis.

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## 1. Introduction

SARS coronavirus-2 (SARS-CoV-2) that emerged as an outbreak in Hubei province of China has now rapidly spread to many parts of the world causing a pandemic. Infection with SARS-CoV-2 results in a pulmonary disease, named Covid-19, which is proving to be a major global health burden [1]. Substantial proportion of cases progress to severe disease, where approximately 5% of patients require intensive care support and over 20% of critical cases succumb to the disease [2]. While overall mortality rate is likely to be lower than the current estimates, as the prevalence of mildly symptomatic cases has yet to be clearly defined, Covid-19 nonetheless represents a global health emergency. There are no licensed vaccines or proven antiviral therapy to protect or treat against Covid-19. Hence once infected, case management is entirely supportive care [2]. Antiviral therapy that effectively arrests disease progression and efficacious vaccines that protect against severe Covid-19 is thus urgently required to meet medical and public health needs.

To meet these needs, various groups have made remarkable strides in bringing new therapeutics and vaccines into clinical development within a very short period of time. A convergence between

therapeutics and vaccines for Covid-19 is the harnessing of the immune response to SARS CoV-2.

A rapidly implementable approach to the development of antivirals is the use of plasma-derived polyclonal hyperimmune globulin. Convalescent Covid-19 patients can be expected to have reasonably high titers of neutralizing antibodies against SARS-CoV-2. Plasmapheresis could be used to collect sufficient volumes of plasma from individuals who have recovered from SARS-CoV-2 infection, pooled and fractionated to produce hyperimmune globulin for infusion into acutely ill Covid-19 patients. Alternatively, fresh frozen plasma could also be delivered without additional fractionation. Such approaches have been explored for patients with viral pneumonia, including SARS and severe influenza [3–5]. Results have been mostly positive although many of such therapies have not been formally evaluated through a randomized, double-blind, placebo-controlled clinical trial. Theoretically, high titers of neutralizing antibody could reduce viral dissemination from infected to uninfected cells in the respiratory tract. When given soon after illness onset, this therapy could thus not only prevent disease progression, it could also lead to more rapid viral clearance and hence patient de-isolation. The latter would enable limited isolation wards to be made available to new Covid-19 patients sooner, enabling management of a larger number of Covid-19 patients despite finite containment capacity.

Under normal circumstances, vaccine development would take years to get from concept to clinical trials. However, lessons from

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research on the related SARS-CoV and MERS-CoV have enabled rapid design of SARS-CoV-2 vaccine candidates for clinical development [6,7]. These vaccines have a common goal, i.e. to elicit polyclonal antibody responses against the spike protein of SARS-CoV-2 to neutralize viral infection. The vaccine candidates encompass a large diversity of vaccine platforms including mRNA, DNA, nanoparticle, subunit, and viral vectors [8]. Some of the new technological platforms, such as DNA and mRNA vaccines, can theoretically enable millions of vaccine doses to be rapidly manufactured within months and deployed to regions of need [9,10]. Indeed, unprecedented in the modern history of infectious diseases and vaccines, a vaccine candidate entered Phase I clinical trials within three months of the discovery of SARS-CoV-2. The World Health Organization (WHO) has announced that a licensed vaccine should be available for widespread use by mid-2021.

While development of both hyperimmune globulin therapy and vaccine against SARS-CoV-2 are promising, they both pose a common theoretical safety concern [11]. Experimental studies have suggested the possibility of immune-enhanced disease of SARS-CoV and MERS-CoV infections, which may thus similarly occur with SARS-CoV-2 infection (as discussed below). The goal of this mini review is thus to examine the evidence for such concerns to guide decision making on therapeutics and vaccines for Covid-19.

Immune enhancement of disease can theoretically occur in two ways. Firstly, non-neutralizing or sub-neutralizing levels of antibodies can enhance SARS-CoV-2 infection into target cells. Secondly, antibodies could enhance inflammation and hence severity of pulmonary disease. An overview of these antibody dependent infection and immunopathology enhancement effects are summarized in Fig. 1. Each of these possibilities would be discussed in turn below.

## 2. ADE of SARS-CoV-2 infection

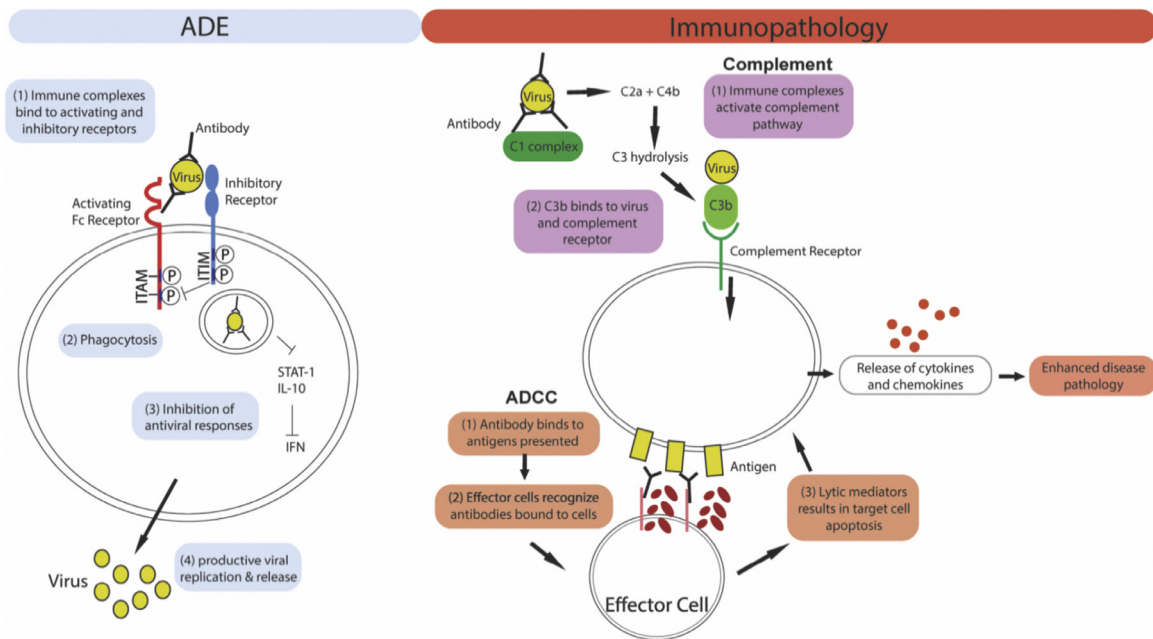
### 2.1. Background

The concern with antibody-dependent enhancement (ADE) of CoV infection arose from observations with feline infectious peritonitis virus (FIPV). FIPV infects myeloid-derived cells, such as macrophages, in cats [12]. As the target cell of FIPV also expresses fragment

crystallizable (Fc) receptors, antibody decorated FIPV could activate Fc receptors for entry into macrophages. Indeed, vaccines that produce low titers of neutralizing antibodies elicited more severe peritonitis and higher mortality rates in vaccinated kittens [13]. Concerns were also raised on the possibility of ADE for SARS-CoV and MERS-CoV infections [14].

### 2.2. The science

ADE of infection can be elicited *in vitro* for many different viruses, including human immunodeficiency virus [15,16], influenza [17] and Ebola viruses [18,19]. Similarly, *in vitro* ADE of wild-type virus and pseudotype viruses into Fc receptor-expressing myeloid-derived cells in the presence of sub-neutralizing concentrations of immune sera has also been described for both SARS-CoV and MERS-CoV [14,20–22]. For CoVs, it has been shown that antibodies can bind the surface spike protein exposing the virus to proteolytic activation and Fc receptor-mediated entry [20]. However, *in vitro* observations need to be interpreted with caution, since few diseases have been clinically associated with ADE. The most prominent disease associated with ADE is arguably dengue, where infection with one serotype of dengue virus (DENV) predisposes a person to a more severe disease upon secondary infection with a heterologous DENV serotype [23,24]. A similar phenomenon was responsible for increased hospitalization rates following vaccination of dengue-naïve individuals with the chimeric tetravalent yellow fever-dengue vaccine, Dengvaxia® [25]. Besides dengue, several other viruses have shown clinical or epidemiological evidence to support the notion of ADE. Two notable examples of vaccine-induced ADE are respiratory syncytial virus (RSV) [26–29] and atypical measles [30,31], where severe disease was more prevalent following vaccination with inactivated virions. Unlike the above-mentioned viral diseases, there is neither clinical nor epidemiological evidence in humans to suggest ADE of CoV infection in severe disease. Re-infection with human CoVs has been observed and there is no report that sequential infection is more severe than primary infection. Likewise, there is also no evidence to suggest that the severity of SARS or MERS is linked to baseline cross-reactive CoV antibodies [32].



**Fig. 1.** Mechanism of ADE and antibody mediated immunopathology. *Left panel:* For ADE, immune complex internalization is mediated by the engagement of activating Fc receptors on the cell surface. Co-ligation of inhibitory receptors then results in the inhibition of antiviral responses which leads to increased viral replication. *Right panel:* Antibodies can cause immunopathology by activating the complement pathway or antibody-dependent cellular cytotoxicity (ADCC). For both pathways, excessive immune activation results in the release of cytokines and chemokines, leading to enhanced disease pathology.

ADE starts when antibody-bound virus binds activating Fc receptors to initiate Fc receptor-mediated endocytosis or phagocytosis. This process facilitates virus entry into Fc receptor-expressing monocytes, macrophages and dendritic cells. However, binding to activating Fc receptors alone is insufficient for ADE. This is because activating Fc receptors trigger signaling molecules that also induce interferon (IFN) stimulated gene (ISG) expression, independent of type-I IFN [33]. ISGs have potent antiviral activities. Consequently, for ADE to occur, viruses must evolve ways to repress such antiviral responses in target cells. For instance, ADE of DENV infection is also dependent on binding of DENV to a co-receptor, the leukocyte immunoglobulin-like receptor B1 (LILRB1) [34]. Signaling from LILRB1 inhibits the pathway that induces ISG expression to create an intracellular environment favorable for viral replication [34–36]. Moreover, we have recently reported that DENV has, in addition to binding LILRB1, also evolved other ways to fundamentally alter the host cell response during antibody-mediated infection, to favor viral replication [37]. Consequently, viruses that exploit ADE must (1) target Fc receptor-expressing cells for infection and (2) have evolved mechanisms to overcome the activating Fc receptor triggered antiviral and other responses in myeloid-derived cells [23]. For viruses to evolve such abilities, Fc receptor-expressing cells must be their primary target so that positive selection can take place. However, currently SARS-CoV-2 has thus far been found to infect angiotensin converting enzyme 2 (ACE2)-expressing epithelial cells [38]. Further studies will be needed to determine the potential of SARS-CoV-2 in infecting myeloid-derived cells [39] and, if any, the role of ADE of SARS-CoV-2 infection in the clinical pathogenesis of Covid-19.

### 3. Antibody-enhanced immunopathology

#### 3.1. Background

Clinical support for antibody-mediated immunopathology comes from the observation that severe SARS disease manifested in week 3 of illness, at a time when respiratory tract viral load was declining due to rising antibody titers [40]. Moreover, Ho and colleagues observed that SARS patients who develop neutralizing antibody responses in the 2nd week of illness were more likely to develop severe disease compared to those who develop antibodies in the 3rd week of illness, or later [32]. A more direct link between antibodies and disease was established in Chinese rhesus macaques, when SARS-CoV-specific antibodies following vaccination or natural infection induced severe pulmonary pathology compared to untreated animals upon viral challenge [41].

#### 3.2. The science

The exact mechanism of antibody-enhanced immunopathology in CoV infection models is not well understood. However, vaccines against viruses such as RSV displayed similar enhanced immunopathology post-vaccination. Antibody-mediated effector pathways have been postulated to be the cause of the enhanced immunopathology [42]. Besides binding to antigen and activating Fc receptor-mediated endocytosis or phagocytosis, antibodies also elicit a number of Fc-mediated responses, namely complement activation and antibody-dependent cellular cytotoxicity (ADCC). Such Fc-mediated effector pathways are generally meant to protect the host by clearing infected cells and recruiting immune cells to sites of infection [43,44]. However, an aberrant, over-stimulated Fc-mediated effector response can also lead to severe immunopathology and damage [42,43]. For example, a study observed that anti-Spike protein IgG antibody from severe SARS patients and from rhesus macaques immunized with a modified Ankara vaccinia vectored SARS CoV vaccine led to the production of proinflammatory cytokines and recruitment of inflammatory macrophages in the lung parenchyma [41]. The exact

mechanism of this immune recruitment is, however, unclear. Hence, a direct link between immunopathology and antibody-dependent complement activation or ADCC during CoV infections has not yet been established.

Despite the temporal relationship between the timings of severe SARS and antibody development [32,40], other possible explanations for the observed pathology have not been systematically excluded. Earlier antibody development could be driven by priming effect from previous human CoV infections with no impact on pathogenesis despite the temporal coincidence with severe disease. Moreover, the kinetics of antibody response is also known to be influenced by viral load [45] and innate immune responses [46]. Higher viral load and elevated cytokine/chemokine expression could thus be the main factors underpinning SARS pathology while an earlier development of neutralizing antibody could be a bystander outcome of those factors.

Pulmonary immunopathology observed in SARS-CoV infection could also be explained by an overwhelming cascade of pro-inflammatory responses triggered by infection-induced cell death. Indeed, infection is known to exacerbate a number of cellular responses, such as the reactive oxygen species (ROS) response arising from cell stress and infection-induced metabolic perturbations, which could drive inflammation [47]. Moreover, cell death would also release purines and pyrimidines, all of which are also known to regulate cellular and immune responses, including inflammation [48,49]. Consequently, temporal association between disease worsening and lowering levels of viral load cannot be completely attributed to antibody-mediated immunopathology.

Finally, a basis to support antibody-mediated disease enhancement is increased activation and recruitment of myeloid-derived cells to the lungs of patients. Indeed, this was the observation in vaccinated macaques, where antibody-mediated pulmonary infiltration by macrophages led to respiratory distress [41]. Histopathological findings from Covid-19 patients, however, show mainly lymphocytic rather than monocytic infiltrates in the lungs of patients who succumbed to acute respiratory distress syndrome [50]. Similarly, serendipitous pathological findings of lung cancer patients who underwent lobectomy at the time when they had undiagnosed Covid-19 did not show significant monocyte or macrophage infiltrates in the alveolar space [51].

### 4. Mitigating risks

Notwithstanding the lack of compelling clinical or histopathological evidence from human Covid-19 cases to support either ADE or antibody-enhanced immunopathology, clinical development of both vaccines and plasma therapy would meet with fewer regulatory hurdles if these risks could be mitigated right at the outset.

#### 4.1. CoV vaccines

Currently, there are multiple SARS-CoV and MERS-CoV vaccine candidates in pre-clinical or early phase clinical trials [6,7]. Animal studies on these CoVs have shown that the spike (S) protein-based vaccines (specifically the receptor binding domain, RBD) are highly immunogenic and protective against wild-type CoV challenge [52]. Vaccines that target other parts of the virus, such as the nucleocapsid, without the S protein, have shown no protection against CoV infection and increased lung pathology [53]. However, immunization with some S protein based CoV vaccines have also displayed signs of enhanced lung pathology following challenge [54–56]. Hence, besides the choice of antigen target, vaccine efficacy and risk of immunopathology may be dependent on other ancillary factors, including adjuvant formulation [55–59], age at vaccination (older mice tend to respond poorly to vaccination) [53,60], and route of immunization [61]. Table 1 summarizes key findings from previous non-clinical studies on various CoV vaccine constructs. Findings from

**Table 1**

Summary of published animal studies reporting protective and immunopathology phenotypes following immunization with various SARS-CoV and MERS vaccines.

Virus	Animal	Vaccine type	Vaccination	Protective <sup>a</sup>	Immuno-pathology <sup>b</sup>	Ref.		
MERS-CoV	Mice	WIV	No Adjuvant	Yes	Yes	[57]		
			Alum	Yes	Yes	[57]		
			MF59	Yes	Yes	[57]		
SARS-CoV	Mice	Adenovirus Vector	S1	Yes	Yes	[63]		
			S1 + CD40L	Yes	No	[63]		
			No Adjuvant	Yes	Yes	[55,58,56]		
		WIV	Alum	Yes	Yes	[55,58 [53],		
			TLR agonist	Yes	<sup>d</sup> Mild	[58]		
			delta inulin adjuvant	Yes	No	[56]		
			No Adjuvant – Aged Mice	Partial	Yes	[53]		
			Alum – Aged Mice	Partial	Yes	[53]		
			VEE Vector	S protein				
				Young mice	Yes	No	[60]	
				Aged mice	Partial	No	[60]	
				N protein				
				Young mice	No	Yes	[60]	
				Aged mice	No	Yes	[60]	
			S + N Protein	Young mice	Yes	<sup>d</sup> Mild	[60]	
		Old mice		No	<sup>d</sup> Mild	[60]		
		VV Vector		S Protein	Yes	No	[76]	
				N Protein	No	Yes	[76]	
				S + N Protein	Yes	Yes	[76]	
		VLP		No Adjuvant	Yes	Yes	[55,77]	
			Alum	Yes	Yes	[55] [77]		
		Subunit	S Protein					
			No Adjuvant	Yes	Yes	[55,56]		
			Alum	Yes	Yes	[55,56]		
			delta inulin adjuvant	Yes	No	[56]		
			TLR agonist	Yes	No	[59]		
			S1 RBD					
<sup>h</sup> FCA Adjuvant	Yes		No	[52]				
No adjuvant	Yes		Yes	[78]				
Feret	WIV	Alum	Yes	Yes	[78]			
		Ad Vector	S + N protein	Yes	Yes	[78]		
			Intra-nasal	Yes	Yes	[78]		
Hamster	MVA Vector	S protein	No	Yes	[54]			
		LAV	Yes	<sup>d</sup> Mild	[79]			
		WIV	No Adjuvant	Yes	<sup>d</sup> Mild	[80]		
AS01	Yes		<sup>d</sup> Mild	[80]				
NHP	MVA Vector	S protein trimer						
		No Adjuvant	Yes	No	[81]			
		Alum	Yes	No	[81]			
		S protein	Yes	Yes	[41]			

<sup>a</sup> Protective - decrease in lung viral titer and/or mortality post-viral challenge.

<sup>b</sup> Immunopathology – histological evidence of damage or cellular (esp. eosinophil) infiltration in the airways.

<sup>c</sup> WIV – Whole Inactivated Virus.

<sup>d</sup> Minor Immunopathology and trace infiltrates observed.

<sup>e</sup> VEE – Venezuelan Equine Encephalitis.

<sup>f</sup> VV – recombinant Vaccinia Virus.

<sup>g</sup> VLP – Virus Like Particle.

<sup>h</sup> FCA – Freund's complete adjuvant.

<sup>i</sup> Ad Vector – Adenovirus Vector.

<sup>j</sup> MVA – Modified Vaccinia Virus Ankara.

<sup>k</sup> LAV – Live Attenuated Virus.

these studies could serve to guide regulatory consideration in accelerating vaccine candidates through clinical development.

Another method of risk mitigation draws upon the lessons learned from cases of vaccine-induced ADE. The vaccines for RSV and measles that could give rise to ADE were both formalin-inactivated vaccines that generated Th2-skewed responses [62]. Similar evidence has been observed in animal studies with CoV vaccine candidates, where vaccines that skew towards a Th1 response elicited protection against both viral infection and immunopathology [56,63]. Vaccines that stimulate Th1 immunity with a strong T cell response component appears to be the way forward. Table 2 summarizes the evidence that T cells may mitigate the risk of immune enhancement in other viral infections. However, an important point of consideration is that the elicitation of T cell responses is not a panacea for vaccine

development, as excessive T cell responses can also result in immunopathology [64,65].

#### 4.2. Hyperimmune globulin therapy

Extending experimental findings of CoV in animal models [41] to infer the risk of antibody-enhanced immunopathology in humans is more nuanced. There are likely major differences between vaccine-induced vs infection-induced antibodies. It is well known that innate immune response drives adaptive immune responses [46,66,67]. As SARS-CoV-2 infection is known to induce cytokine and chemokine expression, convalescent Covid-19 patients would likely produce antibodies that are qualitatively and quantitatively different to those elicited by vaccination alone. Although vaccines could also elicit

**Table 2**  
Mitigation of ADE by T cell responses.

Host	Virus	Model	Protective effect	Ref.
Human	<sup>c</sup> ZIKV	<sup>c</sup> ZIKV-immune individuals stratified by <sup>c</sup> DENV immunity	Broader, greater T cell responses Higher <sup>g</sup> nAb titers Lower ADE	[82]
Human	<sup>d</sup> DENV	<sup>d</sup> DENV ADE on heterologous <sup>d</sup> DENV immune individuals	IFN- $\gamma$ suppresses ADE Addition of anti-IFN- $\gamma$ <sup>g</sup> nAb increases ADE	[83]
<sup>a</sup> AG129 mice	<sup>d</sup> DENV	UV-inactivated <sup>d</sup> DENV + alum vaccination	Transferred CD8 <sup>+</sup> T cells protect mice even under ADE conditions	[84]
<sup>b</sup> A129 mice	<sup>d</sup> DENV	T cell transfer from immunized mice	Transferred CD8 <sup>+</sup> T cells protect mice even under ADE conditions	[85]
Cats	<sup>e</sup> FIPV	Peptide vaccine + CpG adjuvant	Reduced clinical symptoms Improved survival Protection only observed under low dose immunization	[86]
BALB/c mice	<sup>f</sup> RSV	DNA vaccine with additional immunogenic CpG motifs	Higher <sup>g</sup> nAb titers Th1-biased cytokine response	[87]
BALB/c mice	<sup>f</sup> RSV	Adenoviral vaccine vector	Higher <sup>g</sup> nAb titers Th1-biased cytokine response Memory CD8 <sup>+</sup> T cells generated	[88]
BALB/c mice	<sup>f</sup> RSV	DNA vaccine	Higher <sup>g</sup> nAb titers	[89]

<sup>a</sup> *Ifnar*<sup>-/-</sup>*Ifngr*<sup>-/-</sup> mice on 129/Sv genetic background.

<sup>b</sup> *Ifnar*<sup>-/-</sup> mice on 129/Sv genetic background.

<sup>c</sup> Zika virus.

<sup>d</sup> Dengue virus.

<sup>e</sup> Feline Infectious peritonitis virus.

<sup>f</sup> Respiratory syncytial virus.

<sup>g</sup> Neutralizing antibody.

innate immune responses, the magnitude would likely be significantly lower than those found in acutely ill Covid-19 patients since the disease is probably mediated by a pro-inflammatory cytokine response [68]. Moreover, the antigenic burden of wild-type SARS-CoV-2 infection can be expected to be significantly greater than those derived from vaccination. The level of such antigenic burden is known to drive adaptive immune responses, including neutralizing antibody titers [45,69]. Consequently, extending findings from vaccine studies to infer the risk from hyperimmune globulin ignore the possibility of differences between the quality and titer of antibodies produced from infection and vaccination. This same explanation was also found to underpin how inactivated measles vaccination increases the risk of atypical pneumonia [31].

Early anecdotal reports that infusion of convalescent plasma to acutely ill Covid-19 patients suggest the potential of hyperimmune globulin as a treatment to halt the progression of infection to severe pulmonary disease. Hyperimmune globulin treatment, besides inhibiting viral infection, could also downregulate pro-inflammatory responses and reduce disease severity in Covid-19 patients. Intravenous immunoglobulin (IVIG) infusion is associated with anti-inflammatory responses [70], including those from viral infection [71]. Mechanistically, how this anti-inflammatory effect is mediated remains to be fully defined. High dose antibodies could bind a number of different inhibitory receptors, including the inhibitory Fc gamma receptor IIB (Fc $\gamma$ RIIB) [33,72], Fc $\gamma$ RIIC [73,74] or other receptors [75] to induce the anti-inflammatory response. Regardless of the mechanism, the presence of neutralizing SARS-CoV-2 antibodies as well as high concentration of total antibodies could produce anti-inflammatory rather than the postulated immunopathology enhancement effects in Covid-19 patients to improve prognosis.

## 5. Concluding remarks

While experimental evidence to prove or disprove immune enhancement as a pathogenic basis of severe Covid-19 remains to be obtained, current knowledge on the mechanism of ADE and immune enhancement collectively suggest that the risk that hyperimmune globulin or a highly efficacious vaccine pose to exacerbating disease is low. Most descriptions of ADE occur in experimental settings without strong clinical support. Thus, until animal models that accurately capture the pathological features of Covid-19 has been developed and validated, filling the gap in clinical evidence for immune enhancement should be a priority on the research agenda (Box 1).

### Box 1

Investigations needed to further our understanding of the risks of immune enhancement.

The current Covid-19 pandemic with exponential rise in disease cases and deaths urgently calls for both treatment options and efficacious vaccines. However, it cannot be ignored that enhanced immunopathology has been observed with other coronaviruses in animal models, albeit in the absence of convincing clinical evidence. Therefore, in parallel with phase I trials, we suggest urgent clinical evidence to address the gap in clinical evidence for immune enhancement. A list of studies are described below:

- Systematic post-mortem examination with deep immunological and molecular profiling to define the pathology of fatal Covid-19 cases.
- Prospectively enrolled cohort study to determine pre-infection and early post-illness onset immune correlates of Covid-19 disease outcome.
- Develop animal models that replicates the important pathological features found in systematic post-mortem examination of fatal Covid-19 cases.

## 6. Outstanding questions

Large gaps exist in our understanding of the risk of immunopathology with SARS-CoV-2, the epidemiological risk factors, the mechanism and immune mediators of pathology during CoV infections. Clinical studies, preferably in prospectively enrolled cohorts, coupled with detailed investigations into the pre-infection and post-infection correlates of severe Covid-19. Controlled human infection models, perhaps using less virulent CoVs, including human CoV OC43, could also provide a safe avenue to explore the possibility of immune enhancement in CoV infections.

## 7. Search strategy and selection criteria

Data was identified for this review using the following search terms on PubMed: antibody dependent enhancement, ADE, antibody dependent immunopathology, coronavirus, SARS, MERS, SARS-CoV-2, Covid-19, vaccines, treatment, therapeutics, hyperimmune globulin, T cells, antibodies, cross-reactive antibodies. Only articles published in English from 1965 till March 2020 were included.

## Declaration of Competing Interest

The authors declare no conflict of interest.

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