



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Eosinophil responses during COVID-19 infections and coronavirus vaccination



Andrew W. Lindsley, MD, PhD, Justin T. Schwartz, MD, PhD, and Marc E. Rothenberg, MD, PhD *Cincinnati, Ohio*

Eosinophils are circulating and tissue-resident leukocytes that have potent proinflammatory effects in a number of diseases. Recently, eosinophils have been shown to have various other functions, including immunoregulation and antiviral activity. Eosinophil levels vary dramatically in a number of clinical settings, especially following eosinophil-targeted therapy, which is now available to selectively deplete these cells. There are key coronavirus disease 2019 (COVID-19)-related questions concerning eosinophils whose answers affect recommended prevention and care. First, do patients with eosinophilia-associated diseases have an altered course of COVID-19? Second, do patients with eosinopenia (now intentionally induced by biological drugs) have unique COVID-19 susceptibility and/or disease course? This is a particularly relevant question because eosinopenia is associated with acute respiratory deterioration during infection with the severe acute respiratory syndrome coronavirus 2, the causative agent of COVID-19. Third, do eosinophils contribute to the lung pathology induced during COVID-19 and will they contribute to immunopotentiality potentially associated with emerging COVID-19 vaccines? Herein, we address these timely questions and project considerations during the emerging COVID-19 pandemic. (J Allergy Clin Immunol 2020;146:1-7.)

Key words: *Coronavirus, COVID-19, eosinophils, immunopathology, immunopotentiality, SARS, vaccines*

Eosinophils normally account for only a small percentage of circulating leukocytes (1%-3%), but their levels can vary in various disease states.¹⁻³ Their level is clinically relevant because eosinophils are potent proinflammatory cells, primarily due to

Abbreviations used

COVID-19: Coronavirus disease 2019
RSV: Respiratory syncytial virus
S protein: Spike protein
SARS-CoV-1: Severe acute respiratory syndrome coronavirus 1
SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2
TLR: Toll-like receptor

their preformed granules, which are packed with cytotoxic proteins, including major basic protein (one of the most basically charged molecules in the body), eosinophil peroxidase, and 2 RNAses (eosinophil cationic protein and eosinophil neurotoxin). In addition to their proinflammatory effects, evidence is emerging, albeit primarily in mice, that eosinophils have pleotropic roles as regulatory cells involved in protective immunity, including antiviral responses and shaping diverse physiological responses, such as organ development and metabolism. Although eosinophils are normally considered blood cells, they reside in various tissues. Most notably, eosinophils reside in the gastrointestinal tract, which is their primary residence, and the lung, where a population of regulatory eosinophils, which have unique features compared with inflammatory eosinophils, has been identified.⁴

There are a number of diseases associated with eosinophil expansion in which eosinophils are causally related to the disease pathology, such as subsets of moderate and severe asthma. Accordingly, a number of clinically approved biological antibody-based precision therapies are now available that directly target eosinophils, resulting in eosinophil depletion.⁵ These drugs include those that neutralize the eosinophil growth and activating factor IL-5 (eg, mepolizumab and reslizumab) and drugs that directly induce eosinophil depletion by antibody-dependent cellular cytotoxicity (eg, the anti-IL-5 receptor drug benralizumab).⁶ These drugs have remarkable beneficial effects in a growing number of diseases, including asthma, hypereosinophilic syndrome, and eosinophilic granulomatous polyangiitis (formerly known as Churg Strauss syndrome), and additional clinical indications are actively being pursued. As a result, there is now an increasing number of patients with biological drug-induced eosinopenia.⁷ Although patients with abnormally low eosinophil levels (referred to as eosinopenia) might be considered at risk for diseases normally controlled by eosinophils, there have been no major side effects associated with these therapies to date. In addition to biological drug-induced eosinopenia, eosinophil depletion occurs in response to multiple triggers of acute inflammation,⁸ including during sepsis, and multiple studies have consistently shown that low eosinophil levels correlate with poor outcome in critically ill patients.⁹

From the Division of Allergy and Immunology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati.

Disclosure of potential conflict of interest: M. E. Rothenberg is a consultant for Pulm One, Spoon Guru, ClostraBio, Serpin Pharm, Celgene, Astra Zeneca, Allakos, Arena Pharmaceuticals, Guidepoint, Suvretta, and Capital Management; has an equity interest in the first 4 listed; receives royalties from reslizumab (Teva Pharmaceuticals), PEESV2 (Mapi Research Trust), and UpToDate; and is an inventor of patents owned by Cincinnati Children's Hospital. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication April 10, 2020; revised April 17, 2020; accepted for publication April 17, 2020.

Available online April 25, 2020.

Corresponding author: Marc E. Rothenberg, MD, PhD, Division of Allergy and Immunology, Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave, Cincinnati, OH 45229. E-mail: Rothenberg@cchmc.org.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749/\$36.00

© 2020 American Academy of Allergy, Asthma & Immunology

<https://doi.org/10.1016/j.jaci.2020.04.021>

There are now key coronavirus disease 2019 (COVID-19)-related questions concerning eosinophils whose answers affect recommended prevention and care. First, do patients with eosinophilia-associated diseases have an altered course of COVID-19? Second, do patients with eosinopenia have unique COVID-19 disease features? This is a particularly relevant question because eosinopenia has already been reported in patients with acute respiratory deterioration during infection with severe acute respiratory syndrome (SARS) coronavirus (CoV) 2 (SARS-CoV-2), the causative agent of COVID-19.¹⁰ Third, do eosinophils contribute to the lung pathology induced during COVID-19 and will they contribute to adverse events associated with emerging COVID-19 vaccines? Indeed, eosinophil-associated lung pathology is known to occur following certain viral infections (eg, respiratory syncytial virus [RSV]) and importantly is a known complication in previous severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) vaccination studies (see Table I).¹¹⁻¹⁷ On the basis of previous experience with SARS-CoV vaccines, it is expected that COVID-19 vaccines will need careful safety evaluations for immunopotentiality that might increase infectivity and/or eosinophilic infiltration.¹⁸

EOSINOPHIL RESPONSES DURING RESPIRATORY VIRUS INFECTIONS

The role of eosinophils in mucosal immune responses in the respiratory tract has largely focused on the detrimental impact that these cells can have in inflammatory responses due to their potent proinflammatory function. However, preclinical studies (mainly in mice) have shown that eosinophils are equipped with an assortment of molecular tools that enable them to recognize, respond, and orchestrate antiviral responses to respiratory viruses.¹⁹ Human eosinophils express several endosomal Toll-like receptors (TLRs), including TLR3, TLR7, and TLR9, that detect viral microbe-associated molecular patterns.²⁰⁻²² TLR7 enables eosinophils to recognize single-stranded RNA viruses such as coronavirus, and stimulating this receptor in human eosinophils triggers eosinophil cytokine production, degranulation, superoxide and nitric oxide (NO) generation, and prolonged cellular survival.²¹⁻²³ Eosinophil-derived neurotoxin (EDN/RNase2) and eosinophil cationic protein (ECP/RNase3) from human eosinophils reduce infectivity of RSV by a ribonuclease-dependent mechanism.^{24,25} Both human and murine eosinophils produce NO via inducible NO synthase, which can have direct antiviral effects on parainfluenza virus and RSV.^{23,26,27} Eosinophils are able to produce extracellular traps composed of eosinophilic granule proteins bound to genomic and mitochondrial DNA, and murine eosinophils can release these DNA traps in response to RSV infection *in vitro*,²⁸ especially in oxidative lung tissue environments.²⁹ Eosinophils are also capable of quickly mobilizing preformed granule pools of T_H1 cytokines, including IL-12 and IFN- γ , which are important for mounting effective antiviral immune responses.³⁰ In a murine model of allergic asthma, pulmonary eosinophils upregulate MHC-I and CD86 in response to influenza virus infection, where they can directly interact with CD8⁺ T cells and promote the recruitment of virus-specific CD8⁺ T cells into the lungs to enhance antiviral immunity.³¹ Activated murine and human eosinophils also express MHC-II molecules and costimulatory molecules and can function as antigen-presenting cells for viral antigens, leading to T-cell activation and cytokine secretion.^{32,33} IL-5 transgenic mice,

which constitutively overproduce IL-5 and have systemic eosinophilia, have accelerated viral clearance during infection with RSV.²⁶ Conversely, mice genetically engineered to be eosinophil-deficient have lower viral clearance of RSV than do wild-type controls.²⁶ Adoptive transfer of eosinophils from *Aspergillus fumigatus* antigen-sensitized mice into the airways of influenza virus-infected mice decreases viral titers and increases virus-specific CD8⁺ T cells in comparison to that of animals who did not receive eosinophils.³¹ Interestingly, human subjects with asthma were treated with the antieosinophil drug mepolizumab (an anti-IL-5 humanized mAb) or placebo and subsequently challenged with rhinovirus; mepolizumab-treated patients demonstrated significant increases in their rhinovirus viral titers in the upper airway, supporting an antiviral role for eosinophils.³⁴ Although these data substantiate the antiviral potential of eosinophils, the clinical significance of eosinophils in antiviral responses in human disease continues to remain debatable. Patients with eosinophilic asthma have an increased risk for viral-induced asthma exacerbations, and there is mounting evidence that patients with eosinophilic asthma may actually have reduced innate responses against respiratory viruses.³⁵⁻³⁷ Importantly, biologic agents that decrease pulmonary eosinophil levels reduce asthma exacerbations, and patients with asthma treated with these agents have not been reported to have increased viral infections.^{36,38-43} Rosenberg et al⁴⁴ suggested that eosinophils in the respiratory tract might represent a “double-edged sword,” promoting antiviral responses against some respiratory viruses that could become dysregulated during allergic disease given their increased numbers and/or activation status, ultimately resulting in an exaggerated host response that can lead to host tissue damage. The growing number of biologic agents that target eosinophils may be useful tools to help clarify the role eosinophils have in different antiviral responses. Taken together, although preclinical studies have demonstrated antiviral activity for eosinophils, their clinical relevance in immune responses to different respiratory viruses remains unclear and needs further investigation.

EOSINOPHIL RESPONSE IN COVID-19

Rhinovirus, RSV, and influenza virus are common triggers of viral-induced asthma exacerbations, whereas coronaviruses are far less common triggers for acute asthma exacerbations.^{36,45-48} Asthma has not yet been identified as a major risk factor for severity of SARS-CoV-1 infections.⁴⁹ With regard to SARS-CoV-2, Zhang et al¹⁰ recently reported that none of the 140 hospitalized patients with confirmed COVID-19 in a hospital in Wuhan, China, reported asthma or comorbid atopic disease. Another recent review of 548 patients admitted with COVID-19 to another hospital in Wuhan reported only 5 cases of asthma (prevalence of 0.9%), markedly lower than the prevalence of asthma within the adult population in Wuhan (6.4%).⁵⁰ Leukocytosis, with increased absolute neutrophil counts, has been associated with more severe presentations of COVID-19.⁵⁰⁻⁵³ Interestingly, Zhang et al reported that more than half the patients admitted with COVID-19 (53%) had eosinopenia (defined as absolute eosinophil counts $<0.02 \times 10^9$ cells/L) on the day of hospital admission.¹⁰ Similarly, Du et al⁵⁴ reviewed the medical records of 85 fatal cases of COVID-19 and noted that 81% of the patients had absolute eosinophil counts below the normal range (absolute eosinophil counts $<0.02 \times 10^9$ cells/L)

TABLE I. SARS-CoV-1 murine vaccine studies

Study	Vaccine type	SARS-CoV-1 antigen	Adjuvant	Booster rounds	Neutralizing antibodies	Vaccine-induced pathology
Deming et al, ¹¹ 2006	Recombinant viral particle	S protein (VRP-S)	None	1× (3-7 wk after first)	Yes	No
		Nucleocapsid (VRP-N)	None	1× (3-7 wk after first)	No	Yes, severe (lymph + eos)
Du et al, ¹² 2007	Subunit vaccine: S protein RBD	RBD ₃₁₈₋₅₁₀ -hFc*	Initial: Freund's complete adjuvant Boosters: Freund's incomplete adjuvant	3× (every 3 wk ×2, final at 12 mo)	Yes	No
Yasui et al, ¹³ 2008	Recombinant viral particle	Spike (S)	None	None	Yes (9 d after infection)	Yes, mild (neu)
		Nucleocapsid (nuc)	None	None	No (9 d after infection)	Yes, severe (eos + neu)
		Membrane (M)	None	None	No (9 d after infection)	No
		Envelope (E)	None	None	No (9 d after infection)	No
		Nuc + M + E + S	None	None	Yes (9 d after infection)	Yes, severe (eos + neu)
Bolles et al, ¹⁴ 2011	DIV (formalin/UV)	Whole virus	± Alum	1× (2-3 wk after first)	Yes (DIV + alum) (4 d, after infection)	Yes; eos (4 d, after infection)
		Whole virus	± Alum	1× (2-3 wk after first)	ND	Yes; eos + neu + mac (4 d after infection)
		Whole virus	± Alum	1× (2-3 wk after first)	ND	Yes; eos + neu + mac (4 d after infection)
Tseng et al, ¹⁵ 2012	DIV (formalin/UV)	Whole virus	± Alum	1× (4 wk after first)	Yes (2 mo after booster)	Yes; eos (2 d after infection) (reduced + alum)
		Beta propiolactone-inactivated virus (BPV)	± Alum	1× (4 wk after first)	Yes (2 mo after booster)	Yes; eos (2 d after infection) (no difference + alum)
		Subunit vaccine: Full-length S protein	± Alum	1× (4 wk after first)	Yes (2 mo after booster)	Yes; eos (2 d after infection) (reduced + alum)
		Chimeric virus-like particle (VLP)	± Alum	1× (4 wk after first)	Yes (2 mo after booster)	Yes; eos (2 d after infection) (no difference + alum)
Iwata-Yoshikawa et al, ¹⁶ 2014	UV-inactivated whole virus (UV-V)	Whole virus	± Alum	1× (6-7 wk after first)	Yes (before infection and 3 d and 10 d after infection)	Yes; eos, lymph
		Whole virus	± TLR agonists	1× (6-7 wk after first)	Yes (before infection and 3 d and 10 d after infection)	No
Honda-Okubo et al, ¹⁷ 2015	Subunit vaccine: Partially truncated S protein	Spike ^{ΔTM} (S ^{ΔTM})	± Alum	1× (3 wk after first)	Yes (3 d after infection)	Yes, severe eos (6 d after infection)
		Spike ^{ΔTM} (S ^{ΔTM})	± Advax1	1× (3 wk after first)	Yes (3 d after infection)	Yes, mild eos (6 d after infection)
		Spike ^{ΔTM} (S ^{ΔTM})	± Advax2	1× (3 wk after first)	Yes (3 d after infection)	No

DIV, Double-inactivated whole virus; eos, eosinophil; lymph, lymphocyte; mac, alveolar macrophage; ND, not done; neu, neutrophil; RBD, receptor-binding domain; S^{ΔTM}, Spike protein-transmembrane domain deleted; UV, ultraviolet light; VRP, virus replicon particle; VV, vaccinia; V, virus.

Alum, Aluminum salts; TLR agonist [LPS, poly(I:C), poly(U)]; Advax1, delta inulin microparticles; Advax2, delta inulin microparticles and CpG.

*Fusion protein of SARS-CoV-1 RBD (193 amino acids long) and Fc domain of human IgG₁.

at the time of admission. Lymphopenia has also been a common finding in patients with COVID-19,^{10,50,55,56} and blood eosinophil counts correlated positively with lymphocyte counts in both severe and nonsevere cases.¹⁰ Liu et al⁵⁷ also noted eosinopenia at the time of initial presentation in a small cohort of patients who were treated with lopinavir. Notably, eosinophil levels improved in all patients before discharge, suggesting that resolution of eosinopenia may be an indicator of improving clinical status. The pathophysiology for eosinopenia in COVID-19 remains unclear but is likely multifactorial, involving inhibition of eosinophil egress from the bone marrow, blockade of eosinophilopoiesis, reduced expression of chemokine receptors/adhesion factors,^{8,58} and/or direct eosinophil apoptosis induced by type 1 IFNs released during the acute infection.⁵⁹ Importantly, no eosinophil enrichment into the pulmonary tissue has been observed in samples from patients with COVID-19 at early stages of disease⁶⁰ or in postmortem analyses.⁶¹ Moreover, postmortem analysis of lung tissue from a patient who died from COVID-19 demonstrated signs of acute respiratory distress syndrome that was dominated by mononuclear inflammatory infiltrates, mostly lymphocytes.⁶² Consequently, consistent with SARS-CoV-1, asthma or other allergic comorbidities do not seem to increase the risk for poor outcomes with SARS-CoV-2 infections given the current evidence available. Conversely, multiple studies have identified older age, male sex, hypertension, coronary heart disease, and diabetes as being consistent risk factors for severe COVID-19.^{10,50,54-56} The mechanisms for the male predominance of COVID-19 severity are not known, but it is notable that allergic lung responses to RSV have increased severity in male mice by a thymic stromal lymphopoietin (TSLP)-dependent mechanism, and eosinophil-associated diseases such as eosinophilic esophagitis and hypereosinophilic diseases have a predilection for males.⁶³ Taken together, although the current data are limited, there is little indication that eosinophils have a protective or exacerbating role during SARS-CoV-2 infection. Eosinopenia, however, may serve as a prognostic indicator for more severe COVID.

VACCINE-INDUCED IMMUNOPATHOLOGY LINKED TO CORONAVIRUSES

Following the outbreak of the SARS epidemic in late 2002, investigators raced to develop candidate SARS-CoV-1 vaccines. Diverse strategies were tested, including the use of attenuated or inactivated whole CoV particles, DNA-based vaccines, recombinant viral particles, and recombinant subunit vaccines.⁶⁴ Sera from patients convalescing from SARS revealed robust antibody titers against the spike protein (S protein) and the nucleocapsid protein.⁶⁵ Vaccine candidates that induced neutralizing antibodies targeting the S protein demonstrated efficacy in blocking viral replication,⁶⁶ a concept later confirmed by passive antibody transfer studies.^{67,68} Unfortunately, anti-SARS vaccine-associated pathology emerged in early ferret (hepatitis⁶⁹ and pulmonary eosinophilia^{15,69}), cynomolgus monkey (T_H2-type immunopathology with eosinophils^{15,70}), and mouse (pulmonary eosinophilia)¹¹ studies. SARS-CoV-1–driven, eosinophil-associated T_H2 immunopotentiality also occurred with reinfection (green monkey model), suggesting that immune enhancement of CoV-associated disease may be relevant in future outbreaks of heterologous CoVs.⁷¹ Eosinophil-associated disease enhancement following exposure after

vaccination is unfortunately not a new phenomenon. Historical reports from the 1960s link administration of a candidate formalin-inactivated RSV vaccine to severe, eosinophil-associated pulmonary disease following natural infection. This severe eosinophilic pulmonary disease hospitalized most study participants and led to at least 2 deaths.⁷²⁻⁷⁴ Memories of such disease enhancement postvaccination strongly influenced subsequent RSV F protein subunit vaccine development and trial design.⁷⁵ The development of a safe and efficacious SARS-CoV-2 vaccine will require the development of vaccine candidates that take into account the risk of similar vaccine-associated immunopathology.

In the decade following these early observations, a series of mouse studies (see Table I) evaluated the factors driving the observed T_H2-skewed vaccine immunopathology. Two independent studies using recombinant viral particles (Venezuelan equine encephalitis virus or vaccinia) used isolated SARS structural proteins to investigate the source of immunopathology.^{11,13} Nucleocapsid protein vaccination was implicated as a major driver of vaccine-associated pulmonary eosinophilia, although passive transfer of anti-nucleocapsid protein antibody was not sufficient to drive enhanced T_H2 disease, suggesting a possible role for anti-nucleocapsid protein-specific T cells.¹¹ T_H2-mediated disease enhancement was also linked to age, as vaccination of aged mice (>12 months old) with double-inactivated SARS-CoV-1 led to increased morbidity/mortality and accentuated eosinophilic pulmonary disease.¹⁴ Follow-up studies comparing vaccination strategies, vaccine preparations (whole virus/virus-like particles vs subunits vs subunit fragments), boosting strategies and timing, and the inclusion of alum versus other adjuvants (TLR agonists) have yielded variable results. In early studies, chimeric recombinant virus-like particle vaccines displaying only the SARS S protein did not induce eosinophilia.^{11,13} In contrast, isolated S protein subunit vaccines (Spike^{ΔTM} [S^{ΔTM}]) appeared capable of T_H2 immunopotentiality.¹⁵⁻¹⁷ S protein-derived fragments containing just the receptor-binding domain have also been proposed as vaccine antigens, but these vaccine formulations have required more aggressive use of adjuvants and more boosters (3-4 times more) than other approaches.⁶⁴ Investigations into the T_H2 immunopotentiality capacity of these compounds have been limited but reassuring, with 1 study showing no evidence of pulmonary eosinophilia in postchallenge animals¹² and a follow-up study showing balanced T_H1/T_H2 cytokine induction following vaccination.⁷⁶ Some investigators have implicated the inclusion of the T_H2-skewing adjuvant alum in causing the immunopotentiality, and subsequent studies have shown that the inclusion of T_H1-skewing adjuvants with both whole virus and subunit vaccine candidates has attenuated or blocked the development of pulmonary eosinophilia with SARS-CoV-1 challenge.^{16,17} Alternatively, contaminating exogenous proteins from serum-containing media (ie, BSA) in vaccine preparation or viral stocks may explain the observed T_H2 skewing in certain experiments; however, the absence of eosinophilic infiltrates in mock-vaccinated control animals makes this possibility less likely. Overall, the SARS-CoV-1 vaccination literature documents recurrent, postvaccination disease enhancement in diverse vaccine preparations and across multiple animal models; however, this side effect declines with the use of more tightly defined antigens (S protein receptor-binding domain peptide) and the use of T_H1-skewing adjuvants.

TABLE II. Eosinophil responses related to COVID-19

Issue	Likely significance
Atopy-related eosinophilia	Atopy does not appear to have an exacerbating role in COVID-19
Eosinophil antiviral activity	The antiviral activity of eosinophils is unlikely involved in COVID-19 because the antiviral activity of eosinophils has not yet been observed in humans
Biological drug–induced eosinopenia	There are no data to date substantiating any risk for infections following depletion of eosinophils
COVID-19–associated eosinopenia	The eosinopenia associated with COVID-19 is likely a secondary phenomenon and not directly contributing to the disease course
Lung eosinophilia associated with immunopotentialiation by SARS vaccines	Vaccine candidates must demonstrate the absence of eosinophil-associated disease enhancement before widespread deployment

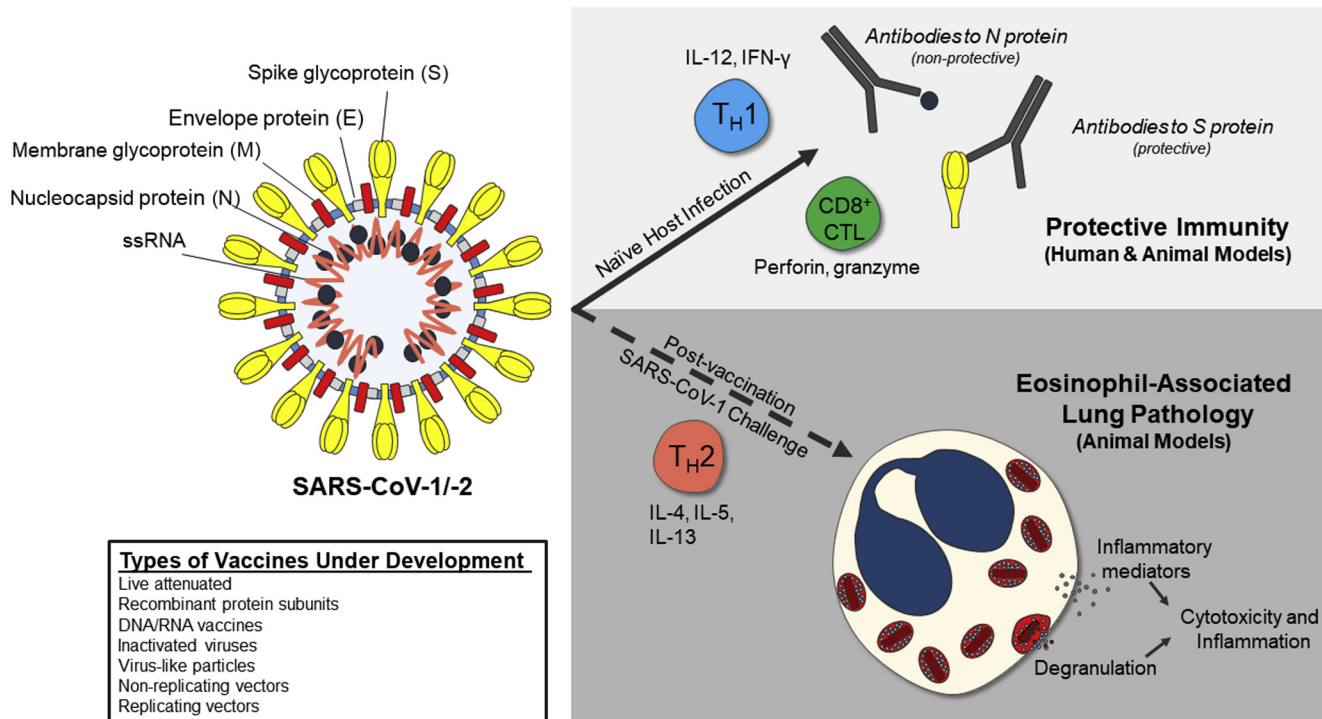


FIG 1. SARS-CoV immunity. The structural proteins of the SARS-CoV viral particle are shown and putative T_H1 - vs T_H2 -mediated immune responses detailed. The Spike (S) glycoprotein mediates binding of the virus to the angiotensin-converting enzyme-2 protein and subsequent fusion/entry into host cells. Sera from convalescing patients have revealed that anti-nucleocapsid protein and anti-S protein antibodies predominate the humoral immune response to SARS-CoV-1 but that only anti-S protein antibodies (especially those targeting the receptor-binding domain region) are neutralizing and protective. Beneficial antiviral responses appear to be linked to T_H1 -skewed immunity, whereas T_H2 immunopotentialiation in multiple animal model systems is associated with vaccination-enhanced disease, leading to pulmonary eosinophilia. To date, these potentially adverse consequences have been observed only in animal model systems following virus challenge with certain vaccine formulations (see Table I). Various SARS-CoV-2 vaccine candidates are currently under development (see box), which should be scrutinized for safety before widespread deployment. CTL, Cytotoxic T lymphocyte; ssRNA, single-stranded RNA.

Conclusions

There are emerging eosinophil-related considerations concerning COVID-19 (Table II). Although current data are limited, there is little supportive evidence that patients with eosinophil-associated diseases will have an altered course of COVID-19, provided that they are not immunosuppressed from concurrent medications or by their primary disease process. Likewise, though preclinical studies have provided compelling experimental evidence that eosinophils have potential antiviral activity, there is no evidence that patients with eosinophilia

induced by the recently approved antieosinophil therapeutics have increased susceptibility to viruses. Whether the acquired eosinopenia associated with COVID-19 is directly contributing to the disease course has not yet been determined, but it is notable that pulmonary eosinophilia is not part of the lung pathology so far attributed to SARS-CoV-2. It will be important however to assess the presence of eosinophils and the deposition of their granule products in the lung of a large cohort of patients with COVID-19, to control for exposure to glucocorticoids, and to determine the role of eosinophils in the COVID-19 lung

pathology. Finally, and likely most importantly, there is considerable concern about whether SARS-CoV-2 exposure postvaccination would cause eosinophil-associated lung pathology through immunopotentiality (see Fig 1). Although these concerns mainly have been derived from murine studies using vaccine candidates from the original SARS-CoV-1 virus, similar responses have also been seen in other species (eg, ferrets and monkey studies); it is also notable that SARS-CoV-1 and SARS-CoV-2 share more than 80% identity. Although the ongoing COVID-19 outbreak places new emphasis on the critical need for an effective SARS-CoV-2 vaccine, safety must be a central focus for any vaccine designed for general use. Current clinical reports show that most (up to 81%) patients with COVID-19 have mild disease,⁷⁷ and therefore, trials of vaccine candidates must rigorously demonstrate the absence of eosinophil-associated disease enhancement before widespread deployment.

Dr Rothenberg thanks the CURED Foundation and Dave and Denise Bunning Fund for kind donations, and the authors thank Shawna Hottinger for editorial assistance.

REFERENCES

- Rothenberg ME. Eosinophilia. *N Engl J Med* 1998;338:1592-600.
- Burris D, Rosenberg CE, Schwartz JT, Zhang Y, Eby MD, Abonia JP, et al. Pediatric hypereosinophilia: characteristics, clinical manifestations, and diagnoses. *J Allergy Clin Immunol Pract* 2019;7:2750-8.e2.
- Schwartz JT, Fulkerson PC. An approach to the evaluation of persistent hypereosinophilia in pediatric patients. *Front Immunol* 2018;9:1944.
- Weller PF, Spencer LA. Functions of tissue-resident eosinophils. *Nat Rev Immunol* 2017;17:746-60.
- Busse W, Chupp G, Nagase H, Albers FC, Doyle S, Shen Q, et al. Anti-IL-5 treatments in patients with severe asthma by blood eosinophil thresholds: indirect treatment comparison. *J Allergy Clin Immunol* 2019;143:190-200.e20.
- Katyal RK, Bensch GW, Busse WW, Chippen BE, Denson JL, Gerber AN, et al. Changing paradigms in the treatment of severe asthma: the role of biologic therapies. *J Allergy Clin Immunol Pract* 2017;5:S1-14.
- Laidlaw TM, Buchheit KM. Biologics in chronic rhinosinusitis with nasal polypsis. *Ann Allergy Asthma Immunol* 2020;124:326-32.
- Bass DA. Behavior of eosinophil leukocytes in acute inflammation. *J Clin Invest* 1975;56:870-9.
- Lavoignet CE, Le Borgne P, Chabrier S, Bidoire J, Slimani H, Chevrolet-Lavoignet J, et al. White blood cell count and eosinopenia as valuable tools for the diagnosis of bacterial infections in the ED. *Eur J Clin Microbiol Infect Dis* 2019;38:1523-32.
- Zhang JJ, Dong X, Cao YY, Yuan YD, Yang YB, Yan YQ, et al. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China [published online ahead of print February 19, 2020]. *Allergy*. <https://doi.org/10.1111/all.14238>.
- Deming D, Sheahan T, Heise M, Yount B, Davis N, Sims A, et al. Vaccine efficacy in senescent mice challenged with recombinant SARS-CoV bearing epidemic and zoonotic spike variants. *PLoS Med* 2006;3:e525.
- Du L, Zhao G, He Y, Guo Y, Zheng BJ, Jiang S, et al. Receptor-binding domain of SARS-CoV spike protein induces long-term protective immunity in an animal model. *Vaccine* 2007;25:2832-8.
- Yasui F, Kai C, Kitabatake M, Inoue S, Yoneda M, Yokochi S, et al. Prior immunization with severe acute respiratory syndrome (SARS)-associated coronavirus (SARS-CoV) nucleocapsid protein causes severe pneumonia in mice infected with SARS-CoV. *J Immunol* 2008;181:6337-48.
- Bolles M, Deming D, Long K, Agnihotram S, Whitmore A, Ferris M, et al. A double-inactivated severe acute respiratory syndrome coronavirus vaccine provides incomplete protection in mice and induces increased eosinophilic proinflammatory pulmonary response upon challenge. *J Virol* 2011;85:12201-15.
- Tseng CT, Sbrana E, Iwata-Yoshikawa N, Newman PC, Garron T, Atmar RL, et al. Immunization with SARS coronavirus vaccines leads to pulmonary immunopathology on challenge with the SARS virus. *PLoS One* 2012;7:e35421.
- Iwata-Yoshikawa N, Uda A, Suzuki T, Tsunetsugu-Yokota Y, Sato Y, Morikawa S, et al. Effects of Toll-like receptor stimulation on eosinophilic infiltration in lungs of BALB/c mice immunized with UV-inactivated severe acute respiratory syndrome-related coronavirus vaccine. *J Virol* 2014;88:8597-614.
- Honda-Okubo Y, Barnard D, Ong CH, Peng BH, Tseng CT, Petrovsky N. Severe acute respiratory syndrome-associated coronavirus vaccines formulated with delta inulin adjuvants provide enhanced protection while ameliorating lung eosinophilic immunopathology. *J Virol* 2015;89:2995-3007.
- Chen WH, Strych U, Hotez PJ, Bottazzi ME. The SARS-CoV-2 vaccine pipeline: an overview. *Curr Trop Med Rep* 2020;1-4.
- Flores-Torres AS, Salinas-Carmona MC, Salinas E, Rosas-Taraco AG. Eosinophils and respiratory viruses. *Viral Immunol* 2019;32:198-207.
- Mansson A, Fransson M, Adner M, Benson M, Uddman R, Bjornsson S, et al. TLR3 in human eosinophils: functional effects and decreased expression during allergic rhinitis. *Int Arch Allergy Immunol* 2010;151:118-28.
- Nagase H, Okugawa S, Ota Y, Yamaguchi M, Tomizawa H, Matsushima K, et al. Expression and function of Toll-like receptors in eosinophils: activation by Toll-like receptor 7 ligand. *J Immunol* 2003;171:3977-82.
- Wong CK, Cheung PF, Ip WK, Lam CW. Intracellular signaling mechanisms regulating toll-like receptor-mediated activation of eosinophils. *Am J Respir Cell Mol Biol* 2007;37:85-96.
- Drake MG, Bivins-Smith ER, Proskocil BJ, Nie Z, Scott GD, Lee JJ, et al. Human and mouse eosinophils have antiviral activity against parainfluenza virus. *Am J Respir Cell Mol Biol* 2016;55:387-94.
- Domachowske JB, Dyer KD, Adams AG, Leto TL, Rosenberg HF. Eosinophil cationic protein/RNase 3 is another RNase A-family ribonuclease with direct antiviral activity. *Nucleic Acids Res* 1998;26:3358-63.
- Domachowske JB, Dyer KD, Bonville CA, Rosenberg HF. Recombinant human eosinophil-derived neurotoxin/RNase 2 functions as an effective antiviral agent against respiratory syncytial virus. *J Infect Dis* 1998;177:1458-64.
- Phipps S, Lam CE, Mahalingam S, Newhouse M, Ramirez R, Rosenberg HF, et al. Eosinophils contribute to innate antiviral immunity and promote clearance of respiratory syncytial virus. *Blood* 2007;110:1578-86.
- Su YC, Townsend D, Herrero LJ, Zaid A, Rolph MS, Gahan ME, et al. Dual proinflammatory and antiviral properties of pulmonary eosinophils in respiratory syncytial virus vaccine-enhanced disease. *J Virol* 2015;89:1564-78.
- Silveira JS, Antunes GL, Gassen RB, Breda RV, Stein RT, Pitrez PM, et al. Respiratory syncytial virus increases eosinophil extracellular traps in a murine model of asthma. *Asia Pac Allergy* 2019;9:e32.
- Yousefi S, Sharma SK, Stojkov D, Germic N, Aeschlimann S, Ge MQ, et al. Oxidative damage of SP-D abolishes control of eosinophil extracellular DNA trap formation. *J Leukoc Biol* 2018;104:205-14.
- Davoine F, Lacy P. Eosinophil cytokines, chemokines, and growth factors: emerging roles in immunity. *Front Immunol* 2014;5:570.
- Samarasinghe AE, Melo RC, Duan S, LeMessurier KS, Liedmann S, Surman SL, et al. Eosinophils promote antiviral immunity in mice infected with influenza A virus. *J Immunol* 2017;198:3214-26.
- Del Pozo V, De Andres B, Martin E, Cardaba B, Fernandez JC, Gallardo S, et al. Eosinophil as antigen-presenting cell: activation of T cell clones and T cell hybridoma by eosinophils after antigen processing. *Eur J Immunol* 1992;22:1919-25.
- Handzel ZT, Busse WW, Sedgwick JB, Vrtis R, Lee WM, Kelly EA, et al. Eosinophils bind rhinovirus and activate virus-specific T cells. *J Immunol* 1998;160:1279-84.
- Sabogal Pineros YS, Bal SM, van de Pol MA, Dierdorp BS, Dekker T, Dijkhuis A, et al. Anti-IL-5 in mild asthma alters rhinovirus-induced macrophage, B-cell, and neutrophil responses (MATERIAL): a placebo-controlled, double-blind study. *Am J Respir Crit Care Med* 2019;199:508-17.
- Bjerregaard A, Laing IA, Backer V, Sverrild A, Khoo SK, Chidlow G, et al. High fractional exhaled nitric oxide and sputum eosinophils are associated with an increased risk of future virus-induced exacerbations: a prospective cohort study. *Clin Exp Allergy* 2017;47:1007-13.
- Edwards MR, Strong K, Cameron A, Walton RP, Jackson DJ, Johnston SL. Viral infections in allergy and immunology: how allergic inflammation influences viral infections and illness. *J Allergy Clin Immunol* 2017;140:909-20.
- Juhn YJ. Risks for infection in patients with asthma (or other atopic conditions): is asthma more than a chronic airway disease? *J Allergy Clin Immunol* 2014;134:247-57, quiz 58-9.
- Bleecker ER, FitzGerald JM, Chanez P, Papi A, Weinstein SF, Barker P, et al. Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting beta2-agonists (SIRIOCO): a randomised, multicentre, placebo-controlled phase 3 trial. *Lancet* 2016;388:2115-27.
- Busse WW, Bleecker ER, FitzGerald JM, Ferguson GT, Barker P, Sproule S, et al. Long-term safety and efficacy of benralizumab in patients with severe, uncontrolled asthma: 1-year results from the BORA phase 3 extension trial. *Lancet Respir Med* 2019;7:46-59.

40. FitzGerald JM, Bleecker ER, Nair P, Korn S, Ohta K, Lommatzsch M, et al. Benralizumab, an anti-interleukin-5 receptor alpha monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma (CALIMA): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 2016;388:2128-41.
41. Jackson DJ, Korn S, Mathur SK, Barker P, Meka VG, Martin UJ, et al. Safety of eosinophil-depleting therapy for severe, eosinophilic asthma: focus on benralizumab. *Drug Saf* 2020;43:409-25.
42. Lugogo N, Domingo C, Chanez P, Leigh R, Gilson MJ, Price RG, et al. Long-term efficacy and safety of mepolizumab in patients with severe eosinophilic asthma: a multi-center, open-label, phase IIIb study. *Clin Ther* 2016;38:2058-70.e1.
43. Winthrop KL, Mariette X, Silva JT, Benamu E, Calabrese LH, Dumusc A, et al. ESCMID Study Group for Infections in Compromised Hosts (ESGICH) Consensus Document on the safety of targeted and biological therapies: an infectious diseases perspective (Soluble immune effector molecules [II]: agents targeting interleukins, immunoglobulins and complement factors). *Clin Microbiol Infect* 2018;24:S21-40.
44. Rosenberg HF, Domachowski JB. Eosinophils, eosinophil ribonucleases, and their role in host defense against respiratory virus pathogens. *J Leukoc Biol* 2001;70:691-8.
45. Arden KE, Chang AB, Lambert SB, Nissen MD, Sloots TP, Mackay IM. Newly identified respiratory viruses in children with asthma exacerbation not requiring admission to hospital. *J Med Virol* 2010;82:1458-61.
46. Johnston NW, Johnston SL, Duncan JM, Greene JM, Kebadze T, Keith PK, et al. The September epidemic of asthma exacerbations in children: a search for etiology. *J Allergy Clin Immunol* 2005;115:132-8.
47. Johnston SL, Pattemore PK, Sanderson G, Smith S, Campbell MJ, Josephs LK, et al. The relationship between upper respiratory infections and hospital admissions for asthma: a time-trend analysis. *Am J Respir Crit Care Med* 1996;154:654-60.
48. Khetsuriani N, Kazerouni NN, Erdman DD, Lu X, Redd SC, Anderson LJ, et al. Prevalence of viral respiratory tract infections in children with asthma. *J Allergy Clin Immunol* 2007;119:314-21.
49. Yin Y, Wunderink RG. MERS, SARS and other coronaviruses as causes of pneumonia. *Respirology* 2018;23:130-7.
50. Li X, Xu S, Yu M, Wang K, Tao Y, Zhou Y, et al. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan [published online ahead of print April 12, 2020]. *J Allergy Clin Immunol*. <https://doi.org/10.1016/j.jaci.2020.04.006>.
51. Liu Y, Du X, Chen J, Jin Y, Peng L, Wang HXH, et al. Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19 [published online ahead of print April 10, 2020]. *J Infect*. <https://doi.org/10.1016/j.jinf.2020.04.002>.
52. Mo P, Xing Y, Xiao Y, Deng L, Zhao Q, Wang H, et al. Clinical characteristics of refractory COVID-19 pneumonia in Wuhan, China [published online ahead of print March 16, 2020]. *Clin Infect Dis*. <https://doi.org/10.1093/cid/ciaa270>.
53. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China [published online ahead of print March 12, 2020]. *Clin Infect Dis*. <https://doi.org/10.1093/cid/ciaa248>.
54. Du Y, Tu L, Zhu P, Mu M, Wang R, Yang P, et al. Clinical features of 85 fatal cases of COVID-19 from Wuhan: a retrospective observational study. *Am J Respir Crit Care Med* 2020;201:1372-9.
55. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med* 2020;382:1708-20.
56. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020;395:1054-62.
57. Liu F, Xu A, Zhang Y, Xuan W, Yan T, Pan K, et al. Patients of COVID-19 may benefit from sustained lopinavir-combined regimen and the increase of eosinophil may predict the outcome of COVID-19 progression. *Int J Infect Dis* 2020;95:183-91.
58. Hassani M, Leijte G, Bruse N, Kox M, Pickkers P, Vrisekoop N, et al. Differentiation and activation of eosinophils in the human bone marrow during experimental human endotoxemia [published online ahead of print January 10, 2020]. *J Leukoc Biol*. doi: 10.1002/JLB.1AB1219-493R.
59. Butterfield JH. Treatment of hypereosinophilic syndromes with prednisone, hydroxyurea, and interferon. *Immunol Allergy Clin North Am* 2007;27:493-518.
60. Tian S, Hu W, Niu L, Liu H, Xu H, Xiao SY. Pulmonary pathology of early-phase 2019 novel coronavirus (COVID-19) pneumonia in two patients with lung cancer. *J Thorac Oncol* 2020;15:700-4.
61. Barton LM, Duval EJ, Stroberg E, Ghosh S, Mukhopadhyay S. COVID-19 autopsies, Oklahoma, USA [published online ahead of print April 10, 2020]. *Am J Clin Pathol*. <https://doi.org/10.1093/ajcp/aqaa062>.
62. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020;8:420-2.
63. Malinczak CA, Fonseca W, Rasky AJ, Ptascinski C, Morris S, Ziegler SF, et al. Sex-associated TSLP-induced immune alterations following early-life RSV infection leads to enhanced allergic disease. *Mucosal Immunol* 2019;12:969-79.
64. Jiang S, Bottazzi ME, Du L, Lustigman S, Tseng CT, Curti E, et al. Roadmap to developing a recombinant coronavirus S protein receptor-binding domain vaccine for severe acute respiratory syndrome. *Expert Rev Vaccines* 2012;11:1405-13.
65. Qiu M, Shi Y, Guo Z, Chen Z, He R, Chen R, et al. Antibody responses to individual proteins of SARS coronavirus and their neutralization activities. *Microbes Infect* 2005;7:882-9.
66. Buchholz UJ, Bukreyev A, Yang L, Lamirande EW, Murphy BR, Subbarao K, et al. Contributions of the structural proteins of severe acute respiratory syndrome coronavirus to protective immunity. *Proc Natl Acad Sci U S A* 2004;101:9804-9.
67. Bisht H, Roberts A, Vogel L, Bukreyev A, Collins PL, Murphy BR, et al. Severe acute respiratory syndrome coronavirus spike protein expressed by attenuated vaccinia virus protectively immunizes mice. *Proc Natl Acad Sci U S A* 2004;101:6641-6.
68. Ishii K, Hasegawa H, Nagata N, Ami Y, Fukushi S, Taguchi F, et al. Neutralizing antibody against severe acute respiratory syndrome (SARS)-coronavirus spike is highly effective for the protection of mice in the murine SARS model. *Microbiol Immunol* 2009;53:75-82.
69. Weingartl H, Czub M, Czub S, Neufeld J, Marszal P, Gren J, et al. Immunization with modified vaccinia virus Ankara-based recombinant vaccine against severe acute respiratory syndrome is associated with enhanced hepatitis in ferrets. *J Virol* 2004;78:12672-6.
70. Haagsmans BL, Boudet F, Kuiken T, deLang A, Martina BE. Protective immunity induced by the inactivated SARS coronavirus vaccine. Presented at: Xth International Nidovirus Symposium Colorado Springs, CO, June 25-30, 2005.
71. Clay C, Donart N, Fomukong N, Knight JB, Lei W, Price L, et al. Primary severe acute respiratory syndrome coronavirus infection limits replication but not lung inflammation upon homologous rechallenge. *J Virol* 2012;86:4234-44.
72. Kapikian AZ, Mitchell RH, Chanock RM, Shvedoff RA, Stewart CE. An epidemiologic study of altered clinical reactivity to respiratory syncytial (RS) virus infection in children previously vaccinated with an inactivated RS virus vaccine. *Am J Epidemiol* 1969;89:405-21.
73. Castilow EM, Olson MR, Varga SM. Understanding respiratory syncytial virus (RSV) vaccine-enhanced disease. *Immunol Res* 2007;39:225-39.
74. Kim HW, Canchola JG, Brandt CD, Pyles G, Chanock RM, Jensen K, et al. Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. *Am J Epidemiol* 1969;89:422-34.
75. Clark CM, Guerrero-Plata A. Respiratory syncytial virus vaccine approaches: a current overview. *Curr Clin Microbiol Rep* 2017;4:202-7.
76. Du L, Zhao G, Chan CC, Li L, He Y, Zhou Y, et al. A 219-mer CHO-expressing receptor-binding domain of SARS-CoV S protein induces potent immune responses and protective immunity. *Viral Immunol* 2010;23:211-9.
77. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention [February 24, 2020]. *JAMA*. <https://doi.org/10.1001/jama.2020.2648>.