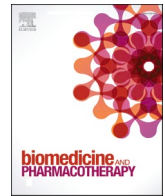




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## Review

# Sterilizing Immunity against COVID-19: Developing Helper T cells I and II activating vaccines is imperative

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## ABSTRACT

Six months after the publication of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) sequence, a record number of vaccine candidates were listed, and quite a number of them have since been approved for emergency use against the novel coronavirus disease 2019 (COVID-19). This unprecedented pharmaceutical feat did not only show commitment, creativity and collaboration of the scientific community, but also provided a swift solution that prevented global healthcare system breakdown. Notwithstanding, the available data show that most of the approved COVID-19 vaccines protect only a proportion of recipients against severe disease but do not prevent clinical manifestation of COVID-19. There is therefore the need to probe further to establish whether these vaccines can induce sterilizing immunity, otherwise, COVID-19 vaccination would have to become a regular phenomenon. The emergence of SARS-CoV-2 variants could further affect the capability of the available COVID-19 vaccines to prevent infection and protect recipients from a severe form of the disease. These notwithstanding, data about which vaccine(s), if any, can confer sterilizing immunity are unavailable. Here, we discuss the immune responses to viral infection with emphasis on COVID-19, and the specific adaptive immune response to SARS-CoV-2 and how it can be harnessed to develop COVID-19 vaccines capable of conferring sterilizing immunity. We further propose factors that could be considered in the development of COVID-19 vaccines capable of stimulating sterilizing immunity. Also, an old, but effective vaccine development technology that can be applied in the development of COVID-19 vaccines with sterilizing immunity potential is reviewed.

## 1. Introduction

Coronaviruses are a group of single-stranded RNA viruses, which affect a wide range of vertebrate hosts [1]. Until about two decades ago when three highly human-pathogenic beta coronaviruses emerged from zoonotic interaction [2], all the four genera of coronavirus (alpha, beta, gamma, and delta) were known to cause only mild upper respiratory tract illnesses in humans [3]. The first highly pathogenic coronavirus severe acute respiratory syndrome-related coronavirus 1 (SARS-CoV-1)

was reported in 2002, where it caused morbidity in about 8000 people with a 10% case fatality [4]. This was followed by the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012, which infected about 2500 people and had a fatality rate of 36% [5]. Currently, the world is faced with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19) [6, 7]. In less than 2 years of pandemic, three variants of the SARS-CoV-2 have emerged [8], each presenting different clinical manifestation of the disease, and thus, resulting in a range of COVID-19 symptoms

**Abbreviations:** SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; COVID-19, coronavirus disease 2019; WHO, World Health Organization; DC's, dendritic cells; ILC's, innate lymphoid cells; PRR's, pattern recognition receptors; PAMPs, pathogen-associated molecular patterns; NF- $\kappa$ B, nuclear factor kappa B; IFN, interferon; ISGs, IFN-controlled genes; URT, upper respiratory tract; CXCL10, Chemokine (C-X-C motif) ligand 10; Th1, T helper 1; Th2, T helper 2; PSO, particle swarm optimization; RBD, receptor-binding domain; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, Immunoglobulin M; LNP, lipid nanoparticles; AdV, adenovirus; NA, nucleic acid; TLR, Toll-like receptors; IVV, inactivated viral vaccine; APC, antigen-presenting cells.

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including but not limited to fever, general malaise and persistent dry cough [9]. The severe form of COVID-19 manifests as acute respiratory distress syndrome and/or acute lung injury that damages the alveolar lumen and causes inflammation and pneumonia [7,10,11]. The severity of COVID-19 and its associated rapid global morbidity and mortality led to a declaration of pandemic on March 11, 2020 by the World Health Organization (WHO) [12]. Thence, knowledge on the morbidity pattern, pathogenesis, and management of COVID-19 has rapidly evolved and same has informed management programs. Like all other infectious diseases, SARS-CoV-2-immune system interaction is critical for the clinical manifestation of COVID-19.

In a matter of six months after the publication of the SARS-CoV-2 sequence, a record number of vaccine candidates were listed, and quite a number of them had begun human trials [13]. In about 18 months into the pandemic, several manufacturers had already published data from clinical trials showing robust efficacy against the disease, and a few had already been approved for emergency use (Table 1). It is worth noting that, a lot more vaccines are getting approval for emergency use as days go by. Also, comprehensive strategies are being employed to ensure vaccination of a significant proportion of the global population, in the quest to achieve herd immunity [14]. An important indicator for a potent vaccine is its ability to safely induce an immune response that recognizes and destroys the pathogen of interest and keeps a memory of that same pathogen such that it can quickly neutralize it upon subsequent exposure. However, data on whether vaccine(s), if any, can confer sterilizing immunity are unavailable. For instance, the efficacy assessment of most of the approved vaccines measured antibody titers [15–18], which largely offer transient protection instead of T cells that can offer protection against future infection and transmission. The Public Health England (PHE) COVID-19 update indicates that, of the 1647 people hospitalized between July 19th and August 2nd, 512 (34.9%) had received two doses of COVID-19 vaccine. This raises concerns about the ability of these vaccines to confer sterilize immunity in recipients. Thus, as important as the regulation of production and distribution of vaccines is, ensuring that approved vaccines can confer sterilizing immunity is also imperative.

A disease causing pathogen must have at least one immune evasion strategy. More important, understanding the immune evasion mechanism of the pathogen is an important requirement to unravel the pathogenesis of the disease it produces [19]. Viruses commonly adopt genome integration and molecular mimicry as strategies to evade immune surveillance, of which SARS-CoV-2 is no exception [20–24]. Even though the first case of COVID-19 was recorded barely a year and a half ago, the scientific advances in understanding SARS-CoV-2 and COVID-19 have been rapid, by any metric. This scientific feat demonstrates the commitment of the scientific community, both academia and industry under extremely challenging conditions. For instance, factors that predispose COVID-19 patients to different degrees of morbidity are fairly understood [7] and the molecular basis of the disease identified [25,26]. The role of genomics in disease diagnoses and treatment [27] has contributed to the discovery of various therapeutic vulnerabilities of COVID-19 [7,28,29].

For pathogens that use molecular mimicry, immune sensitization with part or inactive/semi-active form of the pathogen is usually

effective in interfering with the immune system evasion by the pathogen and disease manifestation [30,31]. However, the duration of immunity is largely dependent on how the vaccine stimulates antibody production or T-cell activation. It should also be noted that immune system activation varies between different individuals following infection. In some individuals, immune response during infection (including SARS-CoV-2) is robust that they neither get sick from the pathogen nor pass the pathogen on to others (sterilizing immunity) [32,33]. While in others, the immune response leads to the production of antibodies that protect them from the disease, but not from future infection of the same pathogen. Even the number and quality of antibodies and/or T-cells that are generated/activated in infection varies between persons, and this knowledge is critical to define treatment and/or immunization strategies. Here, we review the current knowledge of the immunology of COVID-19, with a primary focus on the mechanism of action of some currently approved vaccines, to predict whether they can confer sterilizing immunity (Fig. 1). Further, we propose immunological concepts that could be considered to improve the efficacy of COVID-19 vaccines.

## 2. The immune system and COVID-19

Though the immune system is functionally divided into the innate and the adaptive immune systems, with each consisting of different cell types, they carry out their respective activities in concert.

The innate immune system consists of cells such as macrophages, lymphocytes [34], neutrophils, basophils, dendritic cells (DCs), and innate lymphoid cells (ILCs) [35]. These immune cells reside in tissues or are freely available in circulation and respond to inflammatory stimuli and/or pathogens [36]. In an ideal generic viral infection, the innate immune system triggers a battery of pathogen-borne virulence factors and immune surveillance interactions. This interaction stimulated through the recognition of viral conserved molecular structures known as pathogen-associated molecular patterns (PAMPs) [37], are sensed by the host's germline-encoded pattern recognition receptors (PRRs) [38]. Efficient PAMPs sensing stimulates the host's immune responses in a matter of hours of infection [19] by activating a series of complex signaling pathways climaxed by cytokines and chemokines inflammatory responses [39–41]. These processes culminate in the restriction of viral replication and the creation of an antiviral state in the local tissue of infection, to neutralize the pathogen and primes the adaptive immune system.

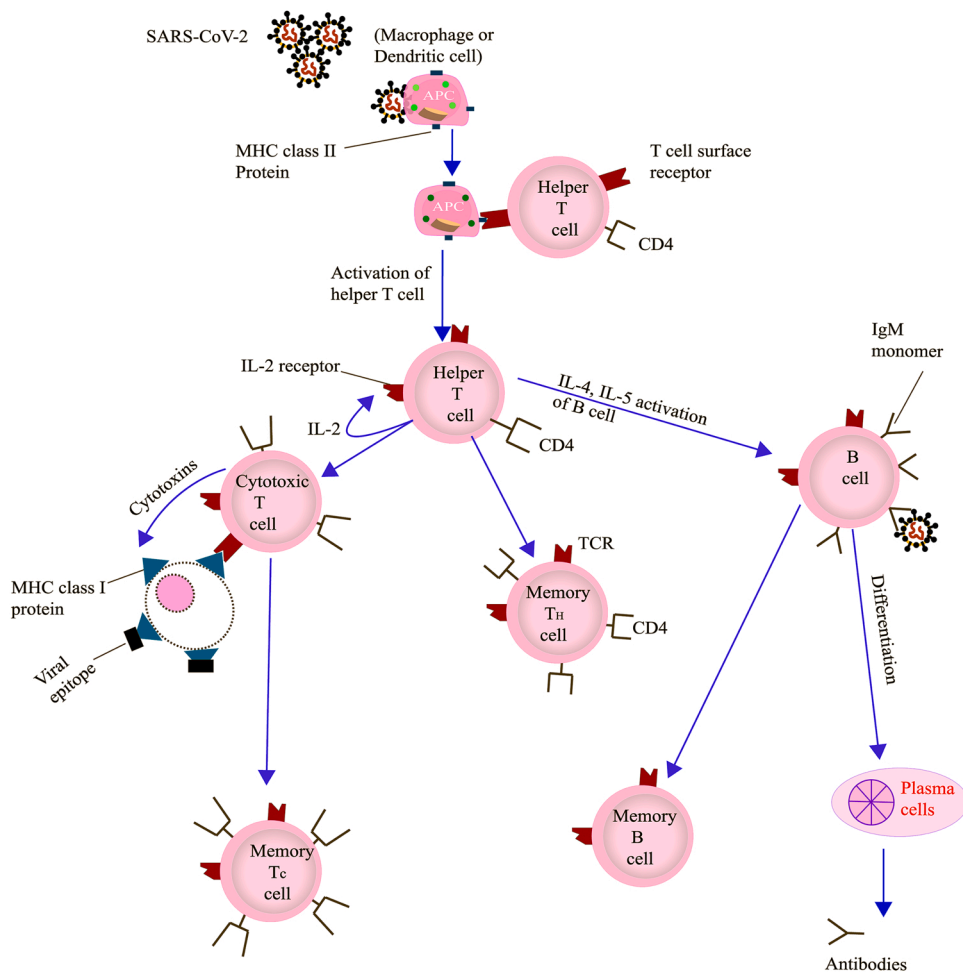
The adaptive immune system is made of three major cell types: CD4 + T cells, B cells, and CD8 + T cells. CD4 + T cells are a range of cells classified as helper T-cells with effector functionalities [19], CD8 + T cells neutralize infected cells through a major histocompatibility class 1 process [42], while the B-cells produce antibodies [43] (Fig. 2). Thus, the adaptive immune response is critical in the control and clearance of SARS-CoV-2, and all viral infections of public health importance. In a viral infection, the primed adaptive immune system differentiates naive B- and T-cells that can recognize viral-specific sequences and molecular structures and proliferate to control the viral infection [19]. The adaptive immune response is inherently slow as it requires between 6 and 10 days after priming to generate sufficient cells interferon regulator factors (IRFs) and nuclear factor  $\kappa$ B (NF- $\kappa$ B) [44].

**Table 1**

List of vaccines approved by the WHO for emergency use.

Company	Date recommended	Shelf Life	Vaccine Name	Dosage	Storage	Efficacy	Target Age-Group
Pfizer/BioNtech	31/12/2020	6 months	BNT162b2 mRNA vaccine	2 doses (21 days apart)	-90°C - -60°C	95%	≥ 12 years
Astrazeneca/SK Bioscience	15/02/2021	6 months	ChAdOx1-S	2 doses (12–14 weeks apart)	2 °C - 8 °C	70.4%	≥ 18 years
Moderna	30/04/2021	7 months	mRNA-1273	2 doses (28 days apart)	-25°C - -15°C	94.1%	≥ 18 years
Johnson and Johnson	12/03/2021	24 months	Ad26-CoV2-S	1–2 doses	-25°C - -15°C	72%	≥ 18 years
Serum Institute India	15/02/2021	9 months	ChAdOx1-S (recombinant)	2 doses	2 °C - 8 °C	78%	≥ 18 years
Sinopharm	07/05/2021	24 months	BBIBP-CorV	2 doses (3–4 weeks)	2 °C - 8 °C	79%	18–59 years
Sinovac Life Sciences	01/06/2021	12 months	CoronaVac	2 doses (2–4 weeks apart)	2 °C - 8 °C	50–84%	≥ 18 years

\*Values of efficacy are shown at preventing symptomatic diseases.



**Fig. 1.** Mechanism of sterilizing immunity activation. The innate immune system detects SARS-CoV-2 viral RNA through IFN signaling and primes the adaptive immune system through antigen presenting cells (APCs). The antigen-APC complex stimulates CD4 positive helper T cells which activates B cells through IL-4 and IL-5 signaling. The B cells proliferate and differentiate into memory B cells and plasma cells that increase antibody production. The CD4 positive helper T cells also differentiate into CD4 positive memory Th cells and stimulate CD8 positive cytotoxic T cells through IL-2 signaling to regulate MHC class I virally infected cells neutralization. Cytotoxic T cells also differentiate into CD8 positive memory Tc cells that together hone memory to SARS-CoV-2 that rapidly clears future SARS-CoV-2 invasion.

IRFs and NF- $\kappa$ B activation lead to the engagement of type I and III interferons (IFN-I and IFN-III)-mediated cellular antiviral defenses that upregulates IFN-controlled genes (ISGs) [45]. Engagement of IFN-I and IFN-III also recruits and coordinates specific subsets of leukocytes [46, 47] and various effector T- cells (helper T cells and cytotoxic T cells) and effector B cells [48] that work in concert to rapidly eliminate the virus.

The SARS-CoV-2 virus has an effective mechanism to avoid the innate immune system, and this phenomenon particularly delays the priming of type I and type III IFNs intracellular innate immune system in humans [49–51]. The delayed stimulation of IFN I and III allows the virus to relentlessly replicate at the early stages of SARS-CoV-2 infection. Often with few exceptions, the temporal delay in triggering IFN I and III responses partly leads to asymptomatic infection [52] or mild disease that does not require hospitalization [19]. Studies elsewhere have also reported that, in patients with acute and convalescent COVID-19 illness, SARS-CoV-2-specific T cell is stimulated earlier than the IFNs and partly associated with milder disease [53–55]. Thus, T cell responses are critical for the resolution of mild COVID-19.

Evidence abounds that ineffective activation of IFN innate immunity results in severe COVID-19, associated with innate cell immunopathology and elevated interleukin (IL)–6, IL-8, and CXCL10 referred to as cytokine storm [56–60]. In immunocompromised individuals and/or successful evasion of the innate immune system, the virus gets unimpeded access during initial replication in the upper respiratory tract (URT) and lungs, leading to the severe form of the disease that requires hospitalization. In adults where there is fewer naïve T-cell pool, the situation is exacerbated as the immune system struggles to make enough SARS-CoV-2-specific T-cells to respond to the viral invasion [54], and thus impedes neutralizing antibody responses. This leads to a high viral

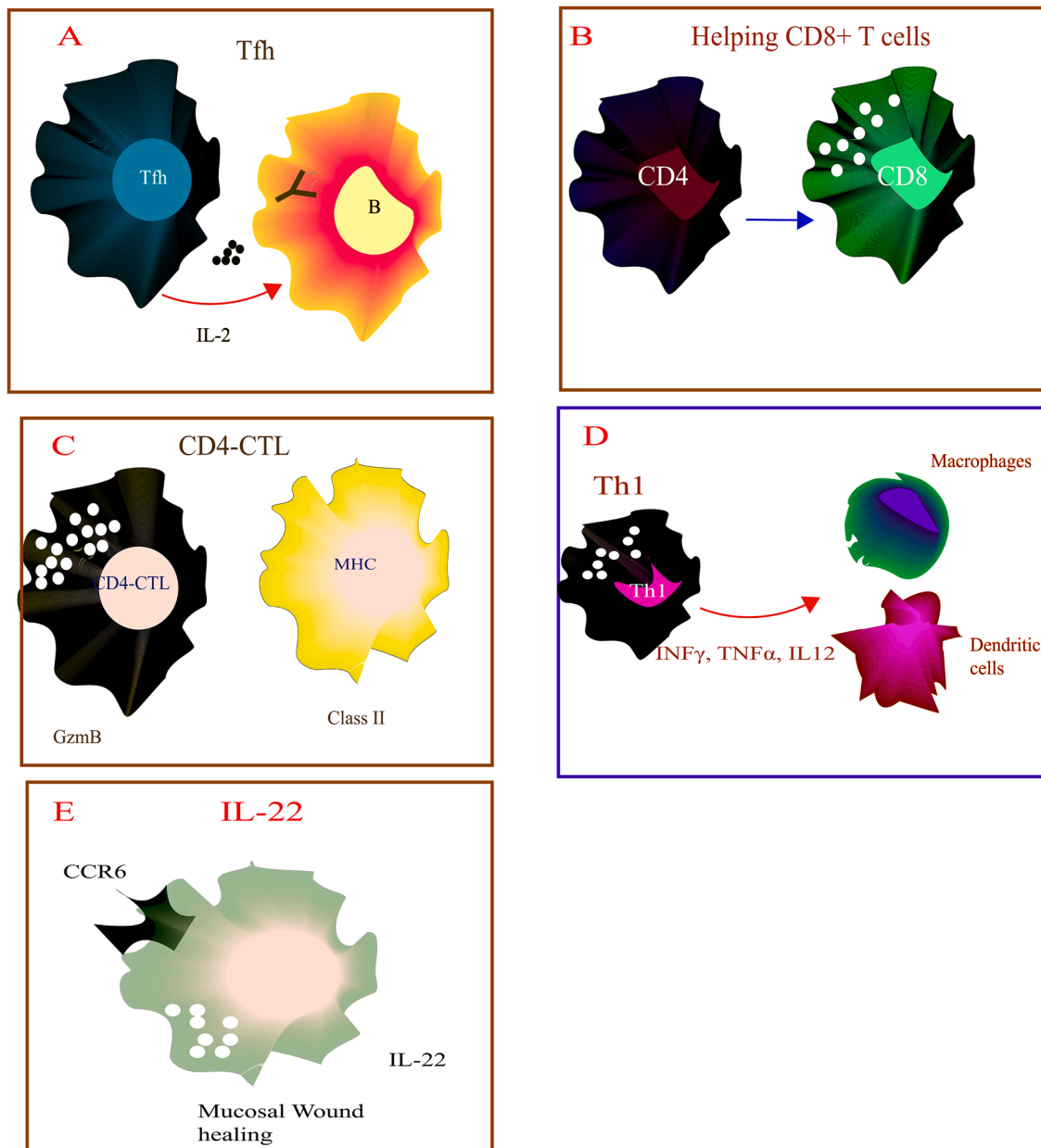
load [61] unmatched by any substantive adaptive immune response. The innate immune system attempts to compensate for the vacuum due to the reduced T cell response, consequently resulting in excessive lung immunopathology [60,62].

### 3. Adaptive immune response to SARS-CoV-2

At the onset of SARS-CoV-2 infection in humans, CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, and antibodies specific to SARS-CoV-2 antigens are generated [63,64]. Each of these adaptive immune cells and antibodies have specific roles which synergistically control the viral infection and ensure host survival.

#### 3.1. CD4<sup>+</sup> T cells

SARS-CoV-2-specific T-cells are detected in and after almost all SARS-CoV-2 infections [54,63,65], and CD4<sup>+</sup> T-cell are the most prominent [63]. CD4<sup>+</sup> T cell response to SARS-CoV-2 infection has also been associated with the effective control of primary infection in humans [54], and in animal models of SARS-CoV-2 infection [66,67]. Grifoni and colleagues reported the propensity for CD4<sup>+</sup> T cells to respond to virion structural protein in convalescence COVID-19 patients. The study found SARS-CoV-2-specific CD4<sup>+</sup> T cells against 21 out of 24 SARS-CoV-2 Spike proteins with negative responses to only the smallest of the proteins [63]. Other studies elsewhere have reported on other viral immune responses, that anti-Spike protein antibodies stimulation largely depends on CD4<sup>+</sup> T cells [68,69]. Thus, SARS-CoV-2 specific T cells response is very crucial in COVID-19 vaccine development, especially in the wake of multiple variants of SARS-CoV-2. This



**Fig. 2.** Functional activity of  $CD4^+$  T cells in COVID-19 immune response. In effective cellular immune response to SARS-CoV-2,  $CD4^+$  T cells differentiate into multiple and distinct cell types that propagate various effector and helper roles. (A) Tfh cells that help B cells for antibody production and affinity maturation; (B) Th1 cells that recruit innate cells and also secrete cytokines to provide direct antiviral functions; (C)  $CD4^+$  T cells also help  $CD8^+$  cells proliferation and differentiation; (D)  $CD4^+$  CTL has direct cytotoxic activity against virally infected cells in MHC class II antigen-presenting cells; (E) Th1 cells secrete  $INF-\gamma$ ,  $TNF-\alpha$  and  $IL-12$  to activate macrophages and dendritic cells; and  $CD8^+$  T cells that produce  $IL-22$  critical in mucosal wound healing.

could explain why there is particular interest in T cell responses against SARS-CoV-2 Spike protein in various COVID-19 vaccines and vaccine candidates [64]. Though the Spike 'M' is a small multipass transmembrane protein without high-affinity class II-restricted T cells in the naïve T cell pool [70], it is reported with nucleocapsid as the most prominent targets of SARS-CoV-2-specific  $CD4^+$  T cells [63], and substantial anti-ORF3a and nsp3 have been reported [63,71–73]. Of note, why Spike 'M' is a prominent target for  $CD4^+$  T cells is still not fully understood.

Even more intriguing is the fact that  $CD4^+$  T cells can differentiate into a range of helper and effector cell types that can stimulate B cells response, complement  $CD8^+$  T cells, exhibit direct antiviral activity, recruit innate cells, and aid tissue repair [19]. Common to this functional differentiation of  $CD4^+$  T cells are T helper 1 cells (Th1) that

produce  $INF-\gamma$  to neutralize virally infected cells [74] and T follicular helper cells (Tfh) that are B cell helpers [75,76]. In mice,  $INF-\gamma^+$   $CD4^+$  T cells have been reported to prevent lethal SARS-CoV infection [67], and it is the most abundant cytokine produced by SARS-CoV-2-specific  $CD4^+$  T cells in COVID-19 patients [54,63,77]. Thus, this functional differentiation of  $CD4^+$  T cells is critical for the development of most neutralizing antibodies, memory B cells, and long-term humoral immunity [78], and in severe COVID-19 where immunopathology is high,  $CD4^+$  T cells also aid  $CD8^+$  T cell responses.

In vaccination, induction of a comprehensive and well-orchestrated immune response that confers sterilizing immunity hinges on optimal and well-guided antibody-inducing B cell and T cell responses [79]. It is established that potent vaccines confer their immunity through antibody production [80], and antibody production heavily relies on the cellular

branch of the immune system. Specifically, Tfh regulates germinal center reaction that induces B cell proliferation, antibody affinity maturation, and B cell differentiation to promote effective antibody responses [81,82]. Evidence abounds that, the production of high titers of neutralizing antibodies in vaccination heavily relies on Tfh cells [83]. These said, the most important function of CD4<sup>+</sup> T cells that is indispensable in immunization is its role in the creation of immune memory, a durable protective immunity that determines disease risk, protection against reinfection, and vaccine efficacy. It is reported that after respiratory viral infection, memory T cells referred to as resident helper T cells (T<sub>RH</sub>) take residence in the lungs to provide a rapid localized immune response during reinfection [84]. Optimal T<sub>RH</sub> formation depends on transcriptional factors that regulate Tfh and resident memory T cell (BCL6 and Bhlhe40) development and provide local help to CD8<sup>+</sup> T cells in an IL-21 dependent mechanism [84].

### 3.2. CD8<sup>+</sup> T cells

In a viral infection, antigen-specific CD8<sup>+</sup> T cells undergo rapid expansion to produce sufficient effector CD8<sup>+</sup> T cells to neutralize the virus through cell-mediated cytolysis and to also produce cytokines to defend the host against rapidly dividing microbe [85]. The expansion is followed by contraction to eliminate about 90% of the effector T cells which restore stable memory cells population. More important, the contraction allows flexibility in response to the multitude of other pathogenic microbes while ensuring enough effective defense against previously exposed ones. Studies elsewhere have shown that, the contraction is a temporal signal to pathogen clearance [86]. Thus, viral load and the duration of infection directly influence the degree and duration of effector T cells. The expression of MHC class I molecules on virtually all nucleated cells facilitates the ability of effector CD8<sup>+</sup> T cells' ubiquitous response against intracellular pathogens. In COVID-19 infection, expansion in SARS-CoV-2-specific CD8<sup>+</sup> T cells have been linked to better COVID-19 outcomes [54,65], though circulating SARS-CoV-2-specific CD8<sup>+</sup> T are not consistently observed as compared to CD4<sup>+</sup> T cells [54,55,63]. SARS-CoV-2-specific CD8<sup>+</sup> T cells have been recorded for Spike, M, ORF3a, and nucleocapsid [54,55,63,65,71,72]. The degree of SARS-CoV-2-specific CD8<sup>+</sup> T cell responses in acute COVID-19 is very much like SARS-CoV-2-specific CD4<sup>+</sup> T cells [54], with evidence of SARS-CoV-2-specific CD8<sup>+</sup> T cells being differentiated as early as day 1 PSO [87].

### 3.3. Antibodies and B cells

The receptor-binding domain (RBD) of SARS-CoV-2 Spike is the prominent target of SARS-CoV-2-specific neutralizing antibodies in COVID-19 [26,88,89]. Studies elsewhere have found that, seroconversion to SARS-CoV-2-specific neutralizing antibodies against Spike range from 91% to 99% [90–92], with Spike IgG, IgA, and IgM developing concurrently in COVID-19 patients [93,94]. In most COVID-19 patients, neutralizing antibodies develop rapidly in the same time frame as seroconversion. SARS-CoV-2 neutralizing antibodies also show virtually no somatic hypermutation [89,95]. The foregoing facts indicate that the synthesis of SARS-CoV-2-specific neutralizing antibodies is rapid and easy. However, the evidence points to the fact that SARS-CoV-2-specific neutralizing antibodies are synthesized from naive B cells, and not from pre-existing cross-reactive memory B cells [96–100]. That said, it is probable that the neutralizing epitopes on the SARS-CoV-2 RBD domain are highly immunogenic making them easily recognizable by antibodies. Of note, circulating SARS-CoV-2-specific neutralizing antibody titers are low in a significantly high number of recovered COVID-19 patients [89, 91,101]. This could mean that neutralizing antibody potency or serum concentration neutralizing antibodies is suboptimal in this subset of recovered patients. In general, and in various animal models, higher antigen load drives higher antibody titers [7].

Naive T cells continue to migrate between the spleen, lymph nodes,

and Peyer's patches via blood and lymph [102]. This makes it impossible for T cells to make contact and/or identify viruses at their point of infection (for example in the lungs for respiratory infections), and only recognize antigens presented to the secondary lymphoid tissues. Nonetheless, most pathogens contain inherent adjuvants such as lipoproteins, unmethylated CpG DNA, lipopolysaccharide (LPS), and double-stranded viral RNA [102]. These inherent adjuvants are immunogenic and thus, are recognized by dendritic cells, and a spectrum of highly conserved, germline-encoded TLRs [103]. Intracellular signaling molecules like Myd88 [104] and ligation of TLRs by adjuvants result in cell activation. Antigen-laden immature dendritic cells migrate through chemotactic induction (CCR1 and CCR5) [105]. Antigen-laden dendritic cells also stimulate the upregulation of T-cells costimulatory molecules such as B7-1 and B7-2 [105] and proinflammatory chemokines and cytokines synthesis [105,106], which guide the migration of activated CCR7 + dendritic cell precursors into the T cell zone. APCs then release TNF $\alpha$  and IL-6, which provide further signals for T cell activation [107]. Given the role of the different branches of the adaptive immune responses in SARS-CoV-2 infection control and immune memory, it is imperative that SARS-CoV-2-specific CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, and antibodies are measured in the same individuals when the efficacy of vaccines are being determined. Such programs can advance frontiers in the engineering of vaccines that can activate the important branches of the adaptive immune system to confer sterilizing immunity on recipients. Whether or not currently approved vaccines can stimulate sterilizing immunity largely depends on how they stimulate the innate immune system and how the adaptive immune system is primed.

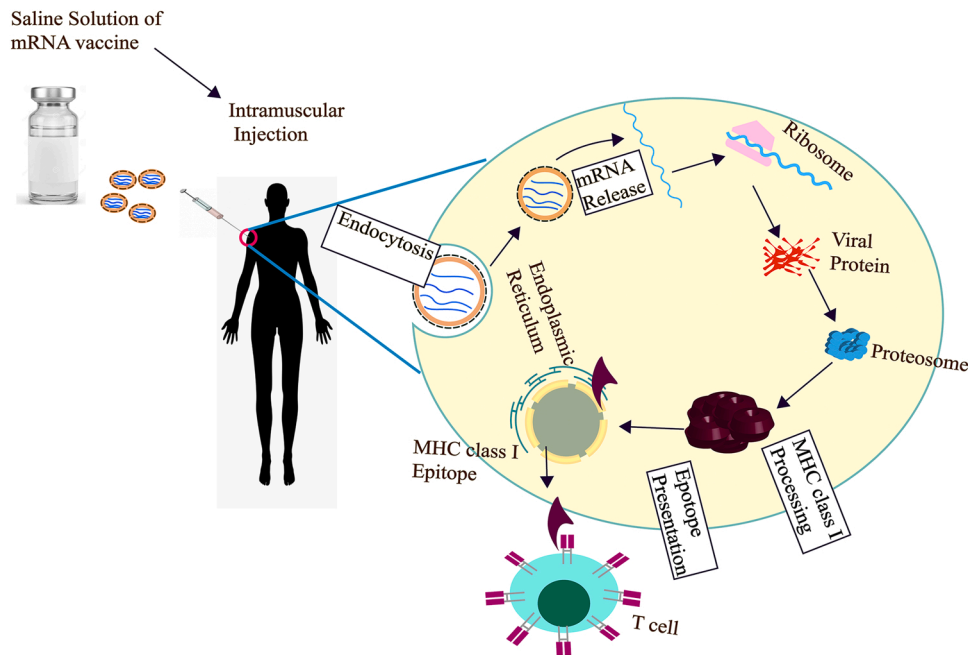
## 4. Vaccine-induced immunity

COVID-19 vaccines can broadly be divided into three; Nucleic acid (NA)-based vaccines, recombinant viral vector vaccines, and inactivated virus vaccines. Pfizer/BioNTech, Moderna, Astrazeneca/Oxford, and Johnson and Johnson produce vaccines that express SARS-CoV-2 spike glycoproteins. However, the Pfizer vaccine (BNT162b2) and Moderna vaccine (mRNA-1273) use mRNA technology and lipid nanoparticle (LNP) delivery systems. Vaccines produced by AstraZeneca (ChAdOx1-S), and Johnson and Johnson (Ad26-CoV2-S) consist of DNA delivered within non-replicating recombinant adenovirus (AdV) vector systems. AstraZeneca uses a chimpanzee adenovirus-expressing spike. Sinopharm uses whole inactivated virus vaccine (BBIBP-CorV) with alum as an adjuvant [108]. Inactivation of the virus is done chemically using beta-propiolactone. Beta-propiolactone binds the viral genes, rendering the viral nucleic acid incapable of replicating.

### 4.1. Nucleic acid-based vaccines

Nucleic acid (NA)-based vaccines are engineered with either DNA or RNA encoding for viral antigenic proteins, using the host's transcriptional and translational machinery to produce viral-specific antigens [109]. If the NA-based vaccine is a DNA-encoding plasmid, the associated genes are transcribed in the nucleus using eukaryotic promoters and translated in the cytoplasm [110]. Conversely, mRNA-based vaccines are transcribed from antigen-coding template DNA by in vitro transcription [110]. Thus, SARS-CoV-2-mRNA vaccines are translated in the cytoplasm to synthesize SARS-CoV-2-antigens which are processed and presented for humoral and T cell immune response activation [111, 112] (Fig. 3). Studies elsewhere have shown that NA vaccines are safe and efficacious for cancer and infectious diseases [110,113,114]. Compared to other vaccine types, NA vaccines can be rapidly developed [115], stimulate both humoral and cellular immune responses, and deliver multiple antigens with single dose [110]. Other studies have also reported on the successful application in various infectious diseases [113,114] and thus, can be engineered to provide sterilizing immunity to COVID-19 [64,114].

The American Biotech company, Moderna's, mRNA-1273 encodes a



**Fig. 3.** Mechanism of action of SARS-CoV-2 mRNA vaccines. SARS-CoV-2 mRNA vaccine is encapsulated in carriers like lipid nanoparticles (LNP) to enhance cellular delivery. The vaccine is administered by intramuscular injection. The LNP-mRNA vaccine enters the host cell by endocytosis and the mRNA is released and translated by the host's protein processing machinery. The resultant protein undergoes proteasome degradation to produce peptides that get associated with MHC class I molecules. The peptides are presented by the host's antigen-presenting cells for recognition by CD8<sup>+</sup> T cells, culminating in the stimulation of cellular immune response against SARS-CoV-2.

prefusion-stabilized SARS-CoV-2 S-protein in lipid nanoparticle capsules. The mRNA-1273, just like the Ad5-nCoV, entered clinical trials before pre-clinical data was released [116]. The phase I clinical data showed that, low and medium doses of two repeated parenteral injections safely induce S protein-specific antibody responses and CD4<sup>+</sup> T cell response [117]. The Pfizer and BioNTech developed BNT162b1 which encode SARS-CoV-2 S protein's RBD encapsulated in lipid nanoparticles have also been reported to stimulate strong S protein-specific antibody and CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses in two repeated parenteral injections [118,119].

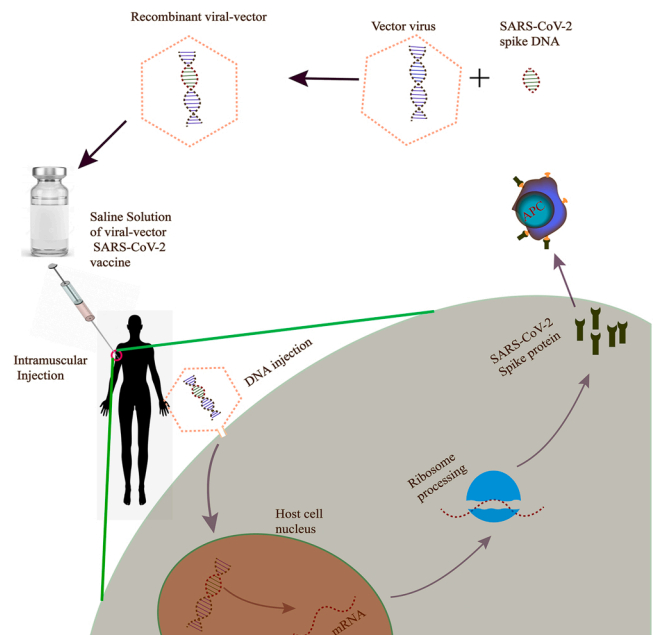
This notwithstanding, the development and clinical application of NA viruses are not without challenges. By far, the most important challenge associated with NA vaccines is how to safely and efficiently deliver them in vivo. This is because most NA-molecules have poor cellular uptake, exhibit nuclease susceptibility, have rapid clearance, and are highly immunogenic, [120–122]. Thus, robust optimization and chemical modifications are required to augment NA vaccine stability and delivery [123]. Often, administration of NA vaccines is done using vector delivery technologies to facilitate their tissue distribution.

Irrespective of the vaccine platform adopted, the resulting prophylactic has two major components: (a) viral-specific immunogen and (b) an adjuvant. For NA vaccines, the viral mRNA encapsulated in LNP usually serves both purposes. This is because the viral encoding protein has intrinsic immunostimulatory properties of RNA. Upon entry into cells, the nucleic acid component of the vaccine is recognized by the endosomal and cytosolic innate immune. NA vaccines stimulate the immune system when endosomal Toll-like receptors (TLR3 and TLR7) binds to single-stranded RNA (ssRNA) in the endosome and NOD2, MDA5, PKR, and RIG-I inflammasomes bind to both double-stranded RNA (dsRNA) and ssRNA in the cytosol. These immune interactions lead to cellular, activation of type I interferon inflammatory mediators [108]. Currently approved NA COVID-19 vaccines mRNA-1273 and BNT162b1 consist of purified in vitro-transcribed ssRNA encapsulated in LNP that has been optimized to reduce its TL-mediated immunostimulatory effect. Thus these vaccines are engineered with reduced type I interferon production and its downstream inhibitory activity on cellular RNA translation [124]. The LNP capsule protects the mRNA and facilitates delivery into specific tissues such as the lymphatic tissue that promote protein translation [118]. In the lymph nodes, dendritic cells engulf the LNP, transcribe the mRNA, and present the antigen to T cells

to stimulate the adaptive immune response.

#### 4.2. Recombinant viral-vector vaccine

Recombinant viral-vectored vaccines are engineered with a viral backbone that is replication-deficient or an attenuated replication-competent but can express the viral-specific antigens (Fig. 4). This vaccine platform has been widely studied for its application in cancer



**Fig. 4.** Basic mechanism of action of recombinant viral-vector SARS-CoV-2 vaccine. DNA fragment encoding for a SARS-CoV-2 antigenic protein such as the "Spike" protein is engineered into the genome of a non-replication adenovirus. Once intramuscularly administered, the DNA encoding the SARS-CoV-2 antigen is integrated into and translated by the host's protein production machinery. The SARS-CoV-2 antigen encoded mRNA is processed by the host's ribosome to produce the "Spike" protein which is sensed and presented by antigen-presenting cells to stimulate a cellular immune response.

and infectious disease, however, very few of those have been approved for use in humans [125]. Among the few is the rVSV-ZEBOV Ebola vaccine [126]. Viral vector vaccines are particularly effective because they are genetically malleable, safe and can induce T cell responses without the need for an adjuvant [127,128]. Another advantage with viral vectors is that they usually need to be administered only once for protection, and the technology for large-scale production already exists [129]. This could explain why recombinant viral-vector vaccine technology is the second most common for COVID-19 vaccine development [130].

The ChAdOx1 nCoV-19 developed by Oxford University, UK, in partnership with AstraZeneca, is a recombinant viral-vector vaccine and the most clinically advanced COVID-19 vaccine. ChAd seroprevalence in humans is very low and accounts for its strong immunogenicity and utility for heterologous prime-boost vaccination against COVID-19 [129, 131,132]. Though the development of viral-vector vaccines is relatively rapid, the unprecedented rapid rate of ChAdOx1 nCoV-19 development hinges on long-term human studies with the ChAdOx1-MERS [133] and ChAdOx1-TB vaccines [134]. Data from phase I and II clinical trials show that ChAdOx1 nCoV-19 induces T cell responses in addition to potent neutralizing antibodies after a single parenteral injection, and this is significantly boosted by a second homologous dose [135]. However, no study has explained the extent to which CD4<sup>+</sup> and CD8<sup>+</sup> T cell subsets are activated. The Ad5-nCOV which is being developed by CanSino Biologics stimulates SARS-CoV-2-specific S protein following intramuscular injection [136]. The announcement that developers had begun phase I and II clinical trials using three dose forms of Ad5-nCOV generated a lot of concerns, because pre-clinical data had not been published. Of particular concern to the international community was that, these doses levels were 10–30 times higher than doses used in previous trials of vaccines with intramuscular administration [137–139]. The concerns were further heightened when the highest dose was withdrawn from the trial in phase II because of unacceptable toxicity [140], and the smaller dose induced S protein-specific neutralizing antibodies in only 50% of the vaccine recipients [141].

The main difficulty associated with the recombinant viral-vector vaccine is that the non-replicating viral platforms are mostly based on Ad5 or MVA, most of which express SARS-CoV-2 specific S protein or RBD. Conversely, replication-competent viral vector vaccines mainly employ the technology of vaccine strains of veterinary pathogens or other human pathogens such as measles or influenza viruses. In circumstances where antibodies pre-exist, because the patient has been exposed to the human or veterinary pathogen on which the current vaccine technology is based, such vaccines are unable to stimulate the immune response. This bottleneck is dealt with by using viral backbones that human exposure is very rare.

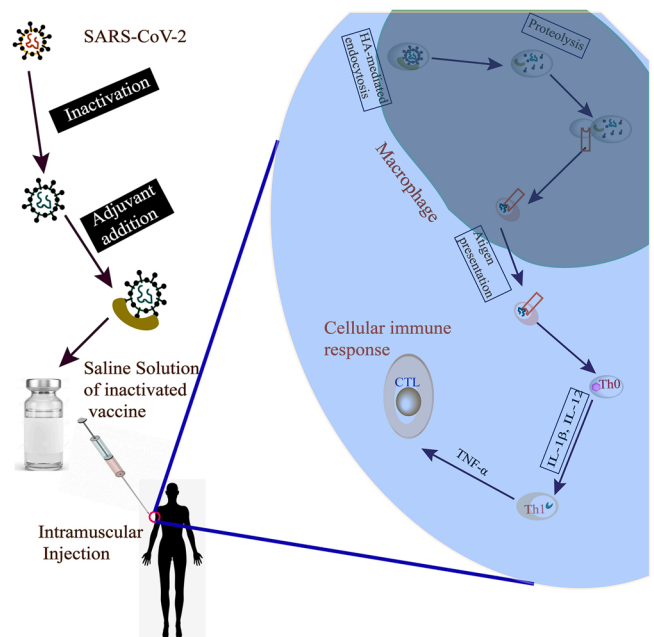
Recombinant viral-vector SARS-CoV-2 vaccines also contain inherent adjuvant properties, incorporated in the virus particle that encapsulates the DNA encoding the viral-specific immunogen [128]. Once administered, AdV particles stimulate the adaptive immune responses through dendritic cells and macrophages, and the innate immune responses by engaging various PRRs. These PRRs particularly TLR9 bind to dsDNA and induce type I interferon secretion [142]. Dendritic cells that take up vaccine-derived nucleic acids encoding the S protein present the viral-specific antigen that delivers inflammatory signals to T cells in the lymph nodes and drains the site of injection. This results in the synthesis of S protein-specific T cells and induction of SARS-CoV-2-specific adaptive immune responses.

#### 4.3. Inactivated viral vaccines

Inactivated virus vaccines (IVV) have a long history of application and success in human diseases such as hepatitis A, Polio, and influenza [143,144]. Its long-term application also suggests that there is state-of-the-art infrastructure available, presupposing that inactivated virus vaccines can be engineered and produced at a pandemic-speed

[145]. Inactivated viral vaccines express a wide range of native viral antigens, and have very few safety concerns [146,147]. Recently, the Sinovac Biotech Ltd in China's alum-adjuvant vaccine has been given approval and global trials in the aged have already commenced [148, 149]. There are reports that, inactivated SARS-CoV vaccines enhance severe disease possibly through a Th2 cell response and lung eosinophilia, [150,151]. Although data from clinical trials did not show that PiCoVacc or BBIBP-CorV worsen lung disease, the alum adjuvant is known to drive Th2 cell-mediated immune responses, and thus, these two vaccines may require further safety investigations [152,153]. The use of Th1 cell-skewing modified alum or other adjuvants such as CpG may avert such safety concerns. It has been reported that intramuscular administration of PiCoVacc or BBIBP-CorV offers some mucosal immunity (Fig. 5), how effective that immunity remains unclear as the SARS-CoV-2 challenge was performed weeks after vaccination [148, 154].

The difficulty associated with inactivated viral vaccines is that, they often require an adjuvant and repeated administration to be effective [153]. The use of alum as an adjuvant [148] makes them unsafe for respiratory mucosal administration [155]. Another significant concern with IVV is associated with ineffective inactivation. In fact, one of the events in the United States Pharmaceutical industry in the history of the United States was the Cutter incident in 1955. The Cutter incident is arguably the worst pharmaceutical catastrophe in the history of the United States. The catastrophe involves 380,000 doses of what was supposed to have been inactivated poliovirus vaccine administered to healthy children. However, viral harvests were contaminated with cell debris due to inadequate purification and this prevented the viral particles from being completely inactivated [156]. Consequently, 40,000 of the children who received the vaccine contracted abortive poliomyelitis, 51 were permanently paralyzed, and 5 died [157]. This led to modification in federal regulations for vaccine manufacturers that would



**Fig. 5.** Basic mechanism of action of inactivated SARS-CoV-2 vaccine. SARS-CoV-2 is cultured, harvested and inactivated to prevent replication while maintaining its antigenicity. An adjuvant such as alum is added to the inactivated vaccine to improve its antigenicity and administered by intramuscular injection. The inactivated SARS-CoV-2 is taken up by antigen-presenting cells such as dendritic cells, which undergo proteolytic degradation to liberate the SARS-CoV-2 antigen. The antigen is sensed by MHC class II and presented to CTL through the Th1-TNF- $\alpha$  signaling pathway to stimulate a cellular immune response.



create a better system for vaccine regulation especially strict protocols that ensure complete inactivation. Aside the Cutter incident, 2 other unfortunate events have been recorded in relation to IVVs. A randomized control trial of a respiratory syncytial virus inactivated with formalin when administered to healthy infants failed to prevent disease, rather 80% of vaccine recipients were hospitalized due to vaccine-induced enhanced disease, compared to 5% hospitalization in the control group [158–160]. It was later discovered that the syncytial vaccine enhanced disease was due to lack of antibody affinity maturation and a skewed Th2 response after vaccination [161,162]. A measles vaccine which was also inactivated with formalin and was licensed in 1963 could not induce neutralizing antibodies. Consequently, recipients became susceptible to measles due to rapid immunity wane out [163].

Today, various inactivation protocols have been described to successfully inactivate viruses for vaccine production that increases safety and efficacy: ethylenimine derivatives [164], ascorbic acid [165], hydrogen peroxide [166], psoralens [167], gamma irradiation [168], heat [169]. Nonetheless, formaldehyde and  $\beta$ -Propiolactone (BPL) are widely used inactivation methods for the production of licensed IVVs for human use.

## 5. Factors worth considering in the development of effective COVID-19 Vaccine

Though NA vaccine platform is relatively new, the technology has the potential of revolutionizing vaccine development to improve efficacy and safety. An important factor that determines the safety and efficacy of a vaccine is how the immunogen is presented to the immune system for recognition. NA vaccine platform provides flexibility for the vaccinologist to determine precisely how the antigen is presented to the immune system for recognition. Sequences can be deleted from or added to the starting cDNA encoding an antigen in its native form to improve the immunogenicity of the final antigen encoded by the resulting NA vaccine. In vaccine development, an efficient delivery system is very critical. Vaccinologist working on the NA platform can remove the intracellular or transmembrane domains to either decrease toxicity or improve solubility [170]. Alternatively, sequences that target the antigen to the MHC class-I or class-II processing pathways can be added [171], to ensure that the T-cell arm of the immune system responsible for the development of sterilizing immunity is stimulated. The issue of whether or not a full-length protein is suitable as a vaccine candidate should be considered and if not, the antigen could be truncated to create minigenes that only encode the immunodominant epitopes, or buried within unrelated, but highly immunogenic core sequences.

NA vaccines are encapsulated in adjuvants to facilitate absorption and effective delivery. Studies should thus, be tailored to the development of immunomodulatory molecules that can augment immunization for use as NA vaccine capsules. For instance, cytokines consisting of limited half-life soluble molecules that can act locally on immune cells can be used as adjuvants to enhance vaccine function [172]. Again, IL-12 can 'steer' the immune response towards the Th1 subset of CD4<sup>+</sup> T cells at the expense of Th2 [172]. Thus, the application of cytokines or cytokine genes as adjuvants can improve the recruitment and maturation of dendritic cells.

In IVVs however, vaccinologists should ensure that viruses that are harvested are completely inactivated. Furthermore, formalin is the commonly used inactivation method and has been found to alter the epitopes which induce functional antibodies required for protection and thus, render such vaccines nonprotective. Further optimization studies should be considered to eliminate any such occurrence.

## 6. The way forward in vaccine development

Currently approved COVID-19 vaccines and vaccine candidates can broadly be divided into inactivated vaccine candidates [173] and immunogenic recombinant vaccine candidates [174]. The inactivated

candidates (PiCoVacc and BBIBP-CorV) are made of attenuated SARS-CoV-2 and induce immunity by mimicking natural SARS-CoV-2 infections without clinical manifestation of COVID-19 [175]. The immunogenic recombinant candidates (Ad5-nCoV, ChAdOx1 nCoV-19, among others.) contain an immunogenic component of SARS-CoV-2 which induces protective immunity [176]. Of the two vaccine candidate types, we recommend the development and use of the immunogenic recombinant candidate type. This vaccine candidate type provides enough room for possible manipulation and engineering for activity optimization. In the development of immunogenic recombinant COVID-19 vaccine, SARS-CoV-2 is grown in laboratory conditions and dissected into various components and their immunogenicity tested. This approach leads to the identification of only SARS-CoV-2 components that can be purified in large quantities for vaccine testing. While this approach has been successful in the development of many vaccines with sterilizing immunity, there are few bottlenecks to their application in obvious immunodominant protective antigens such as SARS-CoV-2 [177], and non-cultivable pathogens. The difficulties associated with this technique is largely due to these three reasons: (a) usually, the most abundant viral proteins are not suitable vaccine candidates, (b) extremely less abundant components are mostly not detected by this technique and even if detected are unable to be tested as vaccines because they cannot be purified in large quantities, and (c) in many cases, not all antigens expressed during infection are expressed during the laboratory cultivation.

Nevertheless, the advent of genomics [178,179], immunotherapy [180–184] and in-silico technology have revolutionized drug discovery in general and vaccine development in particular, especially immunogenic recombinant vaccine candidates. For instance, through genomic analysis, it is now known that the viral genome contains potential antigens which are not necessarily part of the final viral particle. It is also now known that some antigens are present in such low quantities that they cannot be purified in enough quantities and used as antigens by conventional approaches for vaccine development [185]. In this regard, we are proposing a computer-guided genome-based vaccine development approach in reverse vaccinology technology. The whole SARS-CoV-2 genomic sequence is readily available and continues to be updated as new variants emerge. Computer programs could be used to predict all SARS-CoV-2 sequences with antigenic properties and those sequences that are less susceptible to mutation. In-silico technology could then be used to screen for all vaccine candidates regardless of how small. Hits with high fidelity for helper T cell 1 and helper T cell 2 can then be developed into DNA vaccines and expressed as recombinant proteins. The recombinant proteins can then be engineered with bispecific anti-bodies and the efficiency of immunogenicity tested in animal models and subsequently developed into COVID-19 vaccine. This approach has proven effective for HIV vaccine development [186–188] and corroborated the assertion that reverse vaccinology approach could be the most appropriate approach for the development of vaccines for pathogens with no obvious immunodominant protective antigens such as COVID-19 and/or non-cultivable pathogens.

## 7. Conclusion

In the 21st century alone, various coronavirus outbreaks of zoonotic origin have occurred. Among them is the SARS-CoV which occurred between 2002 and 2003, MERS-CoV which began in 2012 and is still ongoing, and the current SARS-CoV-2 which began in 2019. Despite the advances in management programs to interrupt the spread of COVID-19, there are currently no effective therapeutics combating SARS-CoV-2. The development of prophylactics against SARS-CoV-2 has taken the lead in the management programs of the pandemic. While this is good for immediate restoration of normalcy and resumption of full global financial trade and education, intensive research is required to determine whether these vaccines have the prospect to induce sterilizing immunity. Otherwise, equal attention should be given to therapy

development. Following the rapid emergence of SARS-CoV-2 variants, there is the risk of these variants rendering currently approved vaccines ineffective. Therefore, a conscious effort should be made to engineer vaccines that target highly conserved sequences of SARS-CoV-2. In this regard, NA vaccines are better candidates, except that data from clinical trials show better antibody stimulation at the expense of T cell responses.

Though the innate immune system and the adaptive immune system have different functional roles, they work together to provide effective protection during microbial infection. While the innate immune response to infection is rapid, transient, and imminent, the adaptive immune response is delayed. However, once primed by the innate immune system, the adaptive immune system launches a more robust immune response against the infection. While prevention of severe diseases in viral infection largely depends on high titers of neutralizing antibodies, the production of antibodies in itself relies on the CD8 + T-cells to recognize antigen-laden dendritic cells and the CD4 + T-cells to stimulate Th2-mediated antibody production. In particular, the functional differentiation of CD4<sup>+</sup> T cells is critical for the development of most neutralizing antibodies, memory B cells, and long-term humoral immunity that confers sterilizing immunity. It is therefore important that COVID-19 vaccines that have been approved for emergency use demonstrate the capability activate both the helper T-cells I and II pathway of the adaptive immune system.

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#### CRedit authorship contribution statement

**Desmond Omame Acheampong:** Conceptualization, Visualization, Supervision, Writing – review & editing **Isaac Kyei-Barffour:** Writing - original draft, Investigation Visualization **Samuel Addo Akwetey:** Writing - original draft, Investigation **Enoch Aninagyei:** Investigation, Writing – review & editing **George Ghartey-Kwansah:** Investigation, Writing – review & editing.

#### Declaration of Competing Interest

The authors declare no conflict of interest, financial or otherwise.

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