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Nanocarrier vaccine therapeutics for global infectious and chronic diseases

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Immunization has the potential to become a viable weapon for the upcoming pandemic and save millions of lives, while also dramatically lowering the high mortality rate brought on by a number of infectious and chronic illnesses. Despite the success of some vaccinations for infectious illnesses, obstacles remain in avoiding and creating fully protective vaccines. Current COVID-19 pandemic highlights need for vaccination platform improvements. Nanomaterials have been created as a possible nanocarrier to elicit a robust immune response against important global morbidity and mortality drivers by encapsulating targeted antigen and functionalizing nanoparticles with particular molecules. In addition to their application in cancer immunotherapy, nanocarriers are currently being included into the development of vaccines against human immunodeficiency virus (HIV), malaria, TB, and influenza. In order to evaluate conventional and next-generation vaccination platforms, this study focuses on the COVID-19 and cancer vaccine as well as the passage and interaction of nanoparticles with immune cells in the lymph node. It also draws attention to the gaps in current and future HIV, TB, malaria, and influenza vaccinations, as well as nanovaccines. The importance of the dose-dependent vaccine in inducing and maintaining neutralizing antibodies after immunization has been discussed in more detail.

Keywords: Nanovaccine; Infectious diseases; Nanocarriers; COVID-19; HIV; Malaria

Introduction

Immunization is one of the most successful and efficient ways for preventing and controlling infectious diseases in medical research. The first vaccine developed for human use was the smallpox vaccine by Edward Jenner in 1798 [1]. Vaccines have saved millions of lives and significantly reduced economic damage since their introduction over 200 years ago. Whether traditional or next-generation, immunization has substantially contributed to public health throughout history. Traditional vaccines that employ weakened or inactivated microorganisms to induce protection include inactivated, live attenuated, and viral vector vaccines [1]. The vaccine for smallpox was developed using a low-virulence cowpox or horsepox virus that causes only moderate symptoms when exposed to the pathogen [2]. The world's continuously increasing population, along with the emergence of new diseases, necessitates the implementation of effective vaccine platforms.

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Conventional vaccines have certain shortcomings, such as the risk of virulence restoration leading to a poor safety record, the issue of storage, critical administration route, and lower immunogenicity [3]. The poor safety record of the Polio vaccine led to the death of 10 individuals, paralysis of 200, and infection of 40,000 people in 1955. Despite these drawbacks, inactivated vaccines are encouraged because they have a lower risk of causing severe infection [4]. Living pathogenic vaccines are constrained by their need for refrigerated transportation to maintain efficacy and the possibility of reversion to virulent form under certain conditions. Attenuated vaccines do not need to be refrigerated and may be carried in dry form; nevertheless, they cause lower immune responses; thus, booster doses may be required to maintain the effective immunological response [5,6]. At late seasonal flu, antigenic drift, or random genetic mutation of viral strains such as influenza virus gives a difficult time to the formulation of conventional seasonal vaccines to meet the criteria of circulating strain [7-9]. Pathogens such as Ebola [10], tuberculosis causing bacteria *Mycobacterium tuberculosis* [11], antigenically altered HIV [12], and viruses such as SARS-CoV-2 that push us to the limit of global health security and public health [13] are hard-to-target that also demands a greater understanding in working of immunogenicity to develop a vaccine. A potential vaccine has become essential for healthy individuals and, more importantly, older adults who need to restore the immune system to protect themselves from infectious diseases [14]. Immune compromised Individuals have risks associated with live vaccines regarding the safety issue, however, inactivated or killed virus vaccine can be a suitable alternative, but due to lower immunogenicity, they need an adjuvant for enough immune response activation [15].

An advanced and modernized immunization approach is critical to completely eradicate the root of the cause and ensure the control of future pandemics to minimize the mortality rate and economic loss to both developed and developing countries. Although progress has been made in conventional vaccines, improvements are needed due to concerns regarding weak immunogenicity, intrinsic in vivo instability, toxicity, and multi-administration requirements. Emerging technique like nanotechnology is used in next-generation vaccine development for various diseases. Different nanocarriers like lipid-based nanoparticles, liposomes, inorganic nanoparticles (gold, silver), polymer-based nanoparticles, and other carriers made the vaccine delivery easier and more effective [16]. Some nanoparticles are also used as adjuvants to enhance the immune activation. In the current COVID-19 pandemic scenario, major vaccines in the clinical and pre-clinical trials are next-generation vaccines. The total vaccine candidates for COVID-19 include 33% protein subunit vaccines and 19% RNA vaccines, both of which are next-generation vaccines and nanomaterial are being used by several candidates [17]. Future vaccines will not only be limited to preventing infectious diseases but also cancer as a prophylactic and therapeutic tool [18]. Since earlier, nanomaterial is being used in pipelines vaccines, the success of COVID-19 vaccine development encourages more to use nanomaterials as a carrier for HIV, malaria, tuberculosis, and influenzas etc. In this review, we will discuss the drawbacks of traditional and next-generation vaccines and their development and immune response elicitation mechanisms for infectious diseases, notably COVID-19. The nanocarriers employed in next-generation vaccine development, such as nucleic acid-based, subunit, and virus-like particles, will be discussed along with their future outlook.

Role of B-cell and T-cell

Both innate and adaptive immune responses are often termed as two arms of the host defense system. A large number of cells broadly express recognition receptor that recognizes the vaccine antigen or pathogen, initially constitute the innate immune response that rapidly poises the antigen or toxins. After the expression of innate immunity, the adaptive immune response as the second set of response, express itself. A small number of cells, specific to any individual pathogen, compose the adaptive immune system that needs to constantly proliferate after encountering antigens or toxins (from a vaccine or the pathogen) to produce long-lived cells, express effector function, or manifest immune memory. B cells and T cells, the two primary bodies of adaptive immunity, are responsible for humoral and cell-mediated immunity, respectively. The evidence through immunodeficient individuals, immunological data, and passive protection suggest that several functional antibodies with the help of Helper T cells are the major key factor for protection against pathogens induced by vaccination. However, the role of T cell is poorly characterized that it helps the B cell development in the lymph nodes. But it has been clear that T cell helps to control the pathogen hence T cell deficiency can cause uncontrolled proliferation of the pathogen inside the host after infection as studied in the acquired immunodeficient individuals [19]. T cells can be characterized into $CD8^+$ Cytotoxic T cells and $CD4^+$ helper T cells (TH cells). The subtype of Helper T cells, Helper T1 (TH1) and Helper T2 (TH2) are responsible for the establishment of cell-mediated and humoral immune responses, respectively (See Fig. 1). The interleukins they produce are the distinguishable feature of TH cells. Besides, TH1 is also responsible to produce two subclasses IgG1 and IgG3 of the IgG antibody family. TH17 and T follicular cells are other subtypes of T helper cells that are responsible and important for mucosal surface immunity and the generation of high-affinity antibodies [19]. Many mechanisms, including the production of granzymes and perforin, as well as the release of cytokines like interferon (IFN) and tumor necrosis factor, are assisted by CD8+ T cell killing the infected cells [20]. T cells signal B cells to proliferate, alter their antigen receptor class, form a GC, and develop into plasmablasts (the first type of Ab-secreting cells). Within days of infection, plasmablasts grow and produce Abs into the bloodstream and hence throughout the body. These secreted antibodies are the initial and best available response against the pathogen. T cells are developed into specialized T follicular helper (Tfh) cells by B cells, which subsequently control B cell activity throughout the immune response [21]. Most vaccines rely on the production of antibodies to give protection against future infections. The cells are crucial for the establishment of high-affinity B cell clones in the GC and, as a result, for the development of long-term memory cells, such as memory B cells and long-lived plasma cells (LLPCs) [22].

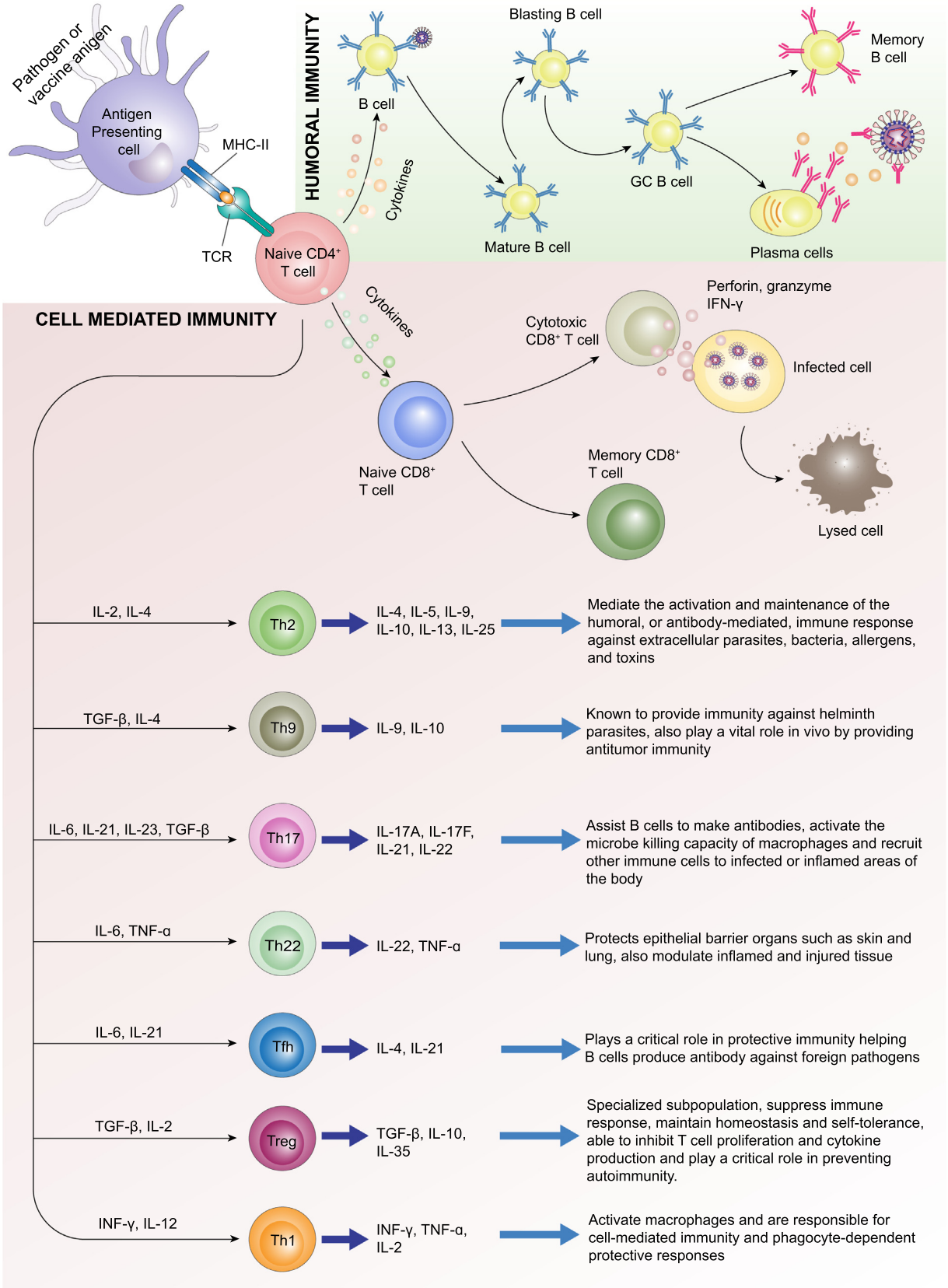


FIGURE 1

Interaction of pathogen or vaccine antigens, induction of humoral and cell-mediated immunity, and different functions of various T cells.

T cells play a significant role in initial response to pathogens and clearing viral infection in the respiratory system by producing a functional memory pool as in the case of COVID-19. The memory T cell generation can protect an individual for years or it can be lifelong. As documented in earlier SARS-CoV and MERS-CoV infections, CD4 and CD8 T cells can be detected in significant proportions after years. Resident memory T cells are short-lived in the lungs, which can ease the blockage of the upper to lower respiratory tract from reinfection. In Influenza A, resident memory T cells has already been demonstrated, but for COVID-19, experiments on animal models can confirm if resident memory T cells can facilitate the rapid control [23]. In addition, as observed in the human study, CD4+ and CD8+ T cells play a significant role in providing immunity from severe influenza infection or fatal outcomes, as well as conferring heterosubtypic immunity. Further, pre-existing CD4+ T cells can reduce influenza symptoms according to a human challenge study [24]. However, it is encouraging to be optimistic about SARS-COV-2 immunity through infection since it has been seen that recovered COVID-19 patients have developed memory B and T cells. Memory B cells could be found after 6 months of infection that can produce neutralizing antibodies, indicating if re-stimulated in secondary infection they can develop into protective antibody-producing plasma cells [21].

Conventional vaccines

Live attenuated vaccine

Live attenuated vaccines (LAVs) are designed from pathogens that have been weakened and are incapable of causing infections [25] where the key to developing LAVs against pathogens, that maintain immunogenicity while exhibiting reduced virulence, is the depletion and modification of pathogenic genes in the viral genome. LAVs have a prolonged immunological memory that triggers both innate and adaptive immunity [26]. Because live attenuated viruses can undergo reversal, choosing a non-pathogenic mutant strain that cannot induce infection is preferable. Even in the compromised state, the attenuated pathogens can elicit robust antiviral immune responses. The initial broadly established approach for developing LAVs is serial viral transmission in Vero cells and chick embryo fibroblasts. With each cycle, the virus's virulence diminishes, this approach has been used to create attenuated viral vaccines for measles, chickenpox, mumps, etc. Apart from this, using the deletion or genetic mutation, resulting in a defective virus incapable of reproduction but capable of eliciting an immunological response in host cells, and incorporating viral proteins into an attenuated cold-adapted virus are major approaches that have been adapted for developing LAVs [27]. Fig. 2 represents the various vaccine platform and the preparation of conventional vaccines.

LAVs can be effective after a single dose causing longer immunogenicity because of the replicating virus. The pathogen-associated signals such as viral RNAs are recognized by Toll-like receptors (TLRs) that induce the innate immune response in cells [28]. Dendritic cells (DCs) express receptors like TLRs to send signals which then target the pathogens. Using multiple TLRs, the dendritic cells are activated in the case of the BCG (Bacillus Calmette-Guerin) vaccine for tuberculosis. In a randomized placebo-controlled study the safety and efficacy of the BCG vaccine against yellow fever were studied that indicated the BCG vaccine to be able to lower the yellow fever viremia [29]. Another live attenuated vaccine YF-17D for yellow fever activates multiple TLRs like TLR-2, TLR-3, and TLR-9. The role of TLRs in adaptive immunity is still unclear. The influenza vaccine which was introduced in the 1960s used only TLR-7 and induced interferon-1 (IFN-1) production by dendritic cells for immunogenicity. In 2004 a live attenuated vaccine for flu was approved which was used for children. Advantages of using these vaccines are their cost-effectiveness because only a small amount is needed for administration which is possible due to a high amount of viral replication [28].

Live attenuated vaccines like MV-014-212 and COVI-VAC for SARS-CoV-2 are in clinical development. MV-014-212 is an investigational vaccine (not yet approved for general use) that is in a phase-1(NCT04798001) clinical trial. The vaccine developed by Meissa vaccines Inc. is a nasal vaccine for the respiratory syncytial virus (RSV) which expresses the spike protein of SARS-CoV-2 [30]. Another live attenuated vaccine developed by Codagenix, and Serum Institute of India is a nasal vaccine COVI-VAC that is in phase-3 (ISRCTN15779782) of clinical trials [31].

Limitations: The major limitations to LAVs are the restoration of virulence caused by compensatory mutations in the genome, recombination, or reassortment [26], and contamination caused during their production. The down-streaming process cannot inactivate the contaminants present because the viral particle in the vaccine is only partially inactivated. For example, in Sabin polio vaccines the human oncogenic virus called SV40 was found as a common contaminant. Another incident occurred in Brazil where the yellow fever vaccine was contaminated with Hepatitis-B virus from 1938 to 1940. There is a chance that even after successful production of the vaccine it may lead to immunosuppression in normal individuals. People with weakened immunity due to chemotherapy or other possible underlying medical conditions could not be given LAVs. Because the weakened pathogens can overwhelm their immune system. The major challenges faced in many countries are because of storage issues as the vaccine contains a live virus, which needs cold storage conditions [28].

Inactivated virus vaccine

Inactivated vaccines (IVs) are produced by killing pathogens using radiation or chemicals, along with turning them incapable of replication and causing infection [25,26]. The mammalian cell line is used to make the inactivated viral stock. Few steps like isolation, sequencing, plaque purification, and passages are followed in viral stock preparation. Then the viral stock is passed through certain chemical agents like ascorbic acid, hydrogen peroxide, formaldehyde, Beta-propiolactone (BPL), and methods like heat, UV treatment, or gamma irradiation. Filtration and chromatography ensure the purity of the sample and cryoelectronic microscopy ensures homogeneity. For better immunogenicity adjuvants like aluminum, and salts are mixed with the final product. After passing through all these steps the virulence and replicating ability of the virus are removed, except for their protective epitopes [28].

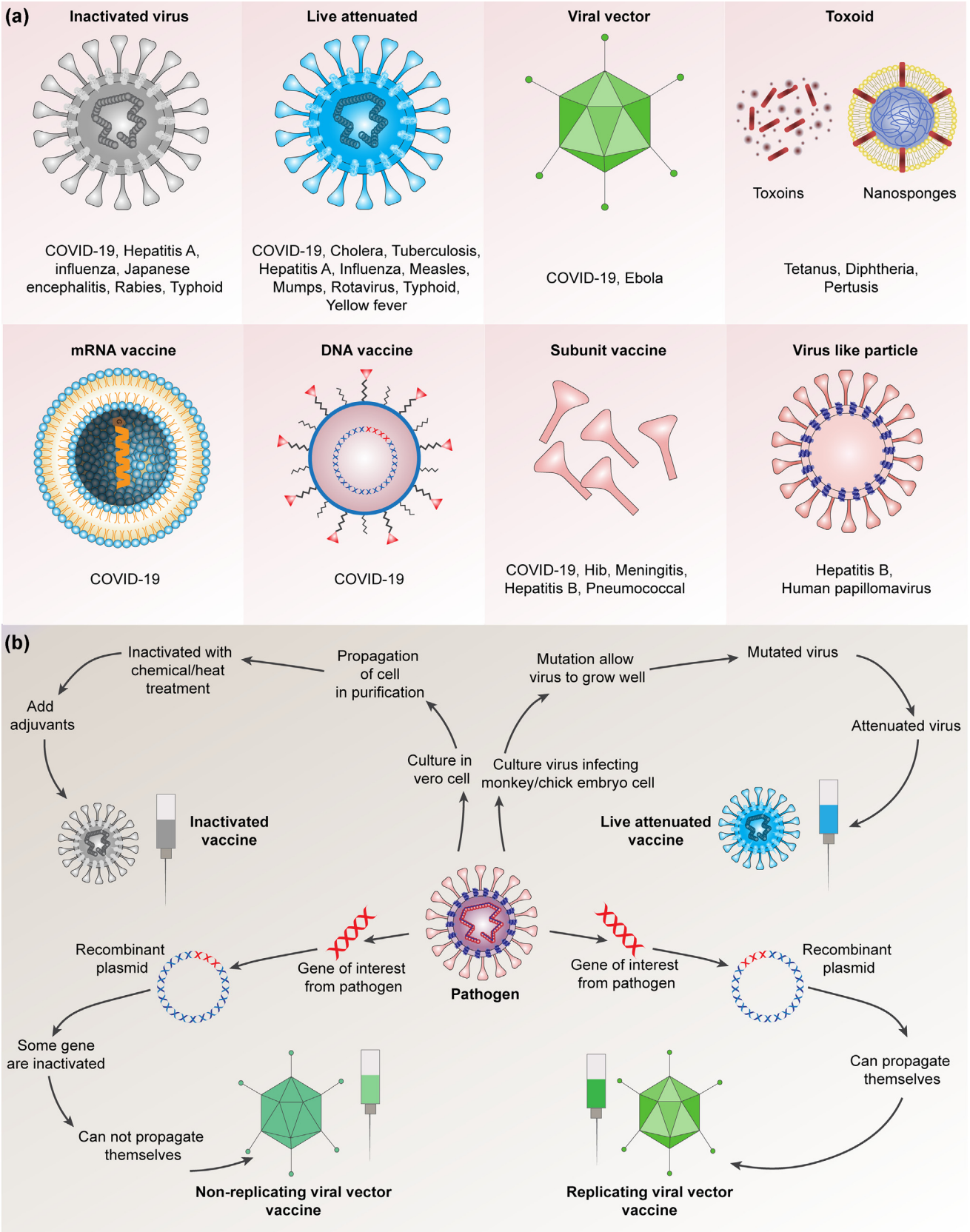


FIGURE 2

(a) Vaccine platforms mentioning their use in different diseases. (b) Flow representation of preparation of inactivated, live attenuated and viral vector vaccine.

Both cell-mediated and humoral immune responses can be triggered by inactivated vaccines. MHC class-1 pathway preserves and cross-presents the constant viral epitopes of CTLs. Immune cell receptors, such as TLR-7, identify viral pathogen-associated molecular patterns (PAMPs) and trigger a T cell-mediated immune response. The production of IFN-1 induces a cell-mediated immune response. For immunosuppressed patients inactivated vaccines are safer to use because they are incapable of causing infections like LAVs [28]. IVs are advantageous over LAVs because of their stability and non-replicating ability.

The IVs developed for SARS-CoV, produced prominent concentrations of neutralizing antibodies against viral S, N, and M proteins [26]. Sinovac Research and development co., China developed a SARS-CoV-2 vaccine CoronaVac, using inactivated virus which is in phase-4 clinical trials [25,26,28]. The clinical trial data of CoronaVac showed that it is effective and safe for both age groups 18–59 and adults aged 60 and above [32,33]. At least 21 Inactivated vaccine candidates are currently in development for SARS-CoV-2. 6 of them are in phase-3 and 3 are in phase-4 clinical trials. Covaxin developed by Bharat biotech international limited is currently in phase-3 trials. A recent study on Covaxin presented its effectiveness against the mutant B.1.1.28 P2 strain. The efficacy of Covaxin is perceived to be around 50.7% in Brazil where the mutant strain is more prevalent. The study also showed that IgG level was increased by administration of two doses of Covaxin leading to the neutralization of SARS-CoV-2 viral particles [34].

Limitations: The inactivated vaccine induces an innate immune response only at the site of injection which makes the administration route critical. Multiple doses are needed for vaccination which makes the administration difficult and cost-effective. Manufacturing of huge amount of viral stock is needed which makes commercial production expensive. Bacterial fermenters, bioreactors, and cell lines are required for the successful production of these inactivated vaccines which requires more time and facilities [28].

Viral vector vaccines

Viral vector vaccines use live repurposed mammalian viruses, which are engineered to deliver a gene encoding one or more antigens into a host cell [25,26]. For therapeutic purposes use of viral vectors, and vaccines started in the late 1990s. Recent studies on dendritic cells (DCs) have shown that this pseudovirus, carrying immunogenicity causing antigens, is non-transferable which causes better stimulation of immunity than recombinant proteins [4].

Viral vectors are of two types: replicating and non-replicating. Replicating or replication-competent vectors develop viruses that infect target cells leading to viral antigen production. Widely used replicating vectors like Measles and Vesicular Stomatitis Virus, contain single-stranded antisense RNA which delivers heterologous antigens inducing a humoral and cell-mediated immune response. Replicating vectors having recombinant form is safer in comparison to the native form. For example, the recombinant Measles Virus Vector (MVV) is used as a multivalent vector for HIV-1 [35] and West Nile Virus [36]. Non-replicating or replication-deficient vectors are sufficient to induce host immune response but cannot replicate inside host cells. Adenovirus-based vectors for instance Ad26 and Ad35 are advantageous for the HIV-1 vaccine that attacks T memory cells [27,37].

Adenovirus vectors (ADVs) are commonly used in gene therapy and vaccine development because of their low virulence, and genetic safety. In the case of SARS-CoV-2 vaccine development vectors like Adenovirus vector type-5 (Ad5) and chimpanzee adenovirus vector (ChAdOx1) are in use [25-27]. Viral vectors like measles virus, vaccinia virus Ankara, and adenovirus vectors (ADVs) loaded with genes encoding N-protein and S-protein trigger anti-coronavirus immunity in a host cell. The viral vectors can produce virus-neutralizing antibodies by CD^{8+} T-cell activation and are proven effective for SARS and MERS. An adenoviral vector developed by Janssen pharmaceutical, and Johnson and Johnson is used against SARS-CoV-2 which was proven effective in rhesus macaques and led to the production of Ad26 vectors containing different SARS-CoV-2 spike protein epitopes. This non-replicating, recombinant adenovirus-based Ad26 vector is used in developing the Ad26.COV2.S vaccine. It encodes the full-length SARS-CoV-2 spike protein and is proven effective for both symptomatic and asymptomatic patients [27,38]. Some leading vaccine candidates like Sputnik V developed by the Gamaleya Research Institute, Russia is using adenoviral vectors like rAd26 and rAd5 carrying SARS-CoV-2 S glycoproteins, rAd26-S and rAd5-S. Results of the clinical trial showed that Sputnik V administration triggered a higher immunogenic response in people because of the presence of antigen-specific $CD4^+$ and $CD8^+$ T cells [27,39]. The phase-3 trial data of Sputnik V showed 91.6% efficacy against Covid-19 [40]. The randomized controlled study of the Ad5-nCoV vaccine which is in phase-4 clinical trials developed by CanSino Biologics Inc. showed that at 5×10^{10} viral particles the vaccine was safe to induce a profound immunogenic response [41]. With the help of genetic engineering, the non-replicating Ad5 vector expresses S-protein. Because the Ad5 vector is a common human serotype, the presence of build immunity by anti-ad5 antibodies in humans can hamper its efficacy and immunogenicity. That is why the clinical utility of the Ad5 vector vaccine is limited and might need an addition of a booster dose [25,26]. The AZD1222 vaccine candidate (using ChAdOx1 vector) developed by AstraZeneca and the University of Oxford is in a phase-4 clinical trial [42,43] and is shown to be effective against the new SARS-CoV-2 variant B.1.1.7. [43].

Mostly the adenovirus vectors are used as delivery systems for vaccine development against rabies, HIV-1, malaria, hepatitis-C virus (HCV), and influenza [28]. The non-replicating chimpanzee adenovirus vector used in rabies vaccine development encodes rabies glycoprotein (G). It belongs to group C, ChAd155-RG, and has proven effective on murine animal models. Clinical trials have shown that they produce neutralizing antibodies in non-human primates [44]. Non-replicating adenovirus type 26 (Ad26) vector is used for Ebola vaccine development. The Ebola vaccine Ad26.ZEBOV encodes Zaire Ebola virus glycoprotein. Another non-replicating, recombinant vaccine MVA-BN-Filo used against Ebola encodes glycoproteins of Zaire Ebola virus, Sudan virus, Marburg virus, and Tai Forest virus nucleoprotein [45]. Along with adenovirus vectors, several Vaccinia virus vectors were used for vaccine development against Smallpox [46],

HIV-1 [47], Cancer [48], Tuberculosis [49], Hepatitis [50] and Malaria [51]. Because of their high transgene expression of different vaccinia viruses, vector-based vaccines can induce a robust immune response against foreign antigens. For Large scale production of Smallpox and HIV-1 vaccines, the vaccinia virus vectors provided evidence of effective immunogenic response induction [46,52].

Limitation: The main limitations of viral vector vaccines are their low immunogenicity and the need to select non or low pathogenic viruses in order to maintain a high biological safety level. Immunogenicity, genetic stability, ability to escape pre-existing immunity, replication deficit or attenuation, genotoxicity, choosing a suitable cell line for propagation, and cost-effectiveness are various factors to consider for developing a viral vector-based vaccine [46].

Toxoid vaccine

Toxoids are employed as antigens in toxoid vaccines, which aid in the protection of bacteria-related diseases. Toxoid vaccines are developed by purifying bacterial exotoxin. In conditions where the toxin is the primary cause of illness, toxoids are formed by inhibiting or inactivating the toxicity of pure exotoxins using heat or formaldehyde without excessive change of the antigenic epitopes and maintaining the immunogenicity. Toxoid vaccination produces anti-toxoid antibodies, which can bind to the toxin leading to its neutralization. Toxins after inactivation are considered harmless and are administered as vaccinations [53]. Because a toxoid is a weakened or inactivated version of the original toxin, the vaccine can induce immunogenicity without causing toxicity or infection. For instance, common bacterial toxin-induced diseases are tetanus, diphtheria, and pertussis. Tetanus is a disease caused by the spore-forming Clostridium tetani bacterium that can be averted with immunizations. Tetanus, which causes muscle spasms and nervous system dysfunction, is still a problem in both developed and developing countries. Few treatment options like wound debridement, use of antitoxins and antibiotics, and proper supportive care provision are used to control the spasm and cardiovascular instability [54,55].

In 1924, a tetanus vaccine was developed which was ineffective. Later, during World War II in the 1940s, a superior version of the vaccine was developed, and it was able to lower the risk of tetanus by 95%. Since then, different classes of tetanus toxoid vaccines like DTP, DTaP, TdaP, Td, and DT were developed, and still, continuous effort is being given towards the development [56,57]. A trivalent vaccine for Diphtheria, Pertussis, and Tetanus named DPT is administered in children below age 7 [57,58], as a part of the neonatal immunization schedule in many countries.

A study was conducted to confirm the potency of a current vaccine against tetanus. The crude chemically inactivated tetanus toxoid vaccine (CITT) is proven to be clinically effective against the tetanus toxoid (TT), but it contains clostridium tetani proteins in variable amounts. An alternative recombinant vaccine 8MTT against tetanus in development made progress by developing a full-length tetanus toxin in a genetically inactivated form. The result of a pilot study confirmed the effectiveness of the 8MTT vaccine in mice. The mice vaccinated with CITT and 8MTT substantiate a difference in the survival duration against TT. The 8MTT vaccinated mice lived longer than the CITT vaccinated mice. This proved that 8MTT is a potential candidate that can act as a conjugate vaccine against TT to induce an immune response to different pathogenic macromolecules like polysaccharides [59,60].

The effectiveness of RBD-Tetanus toxoid-based conjugate vaccine SOBERANA02 developed by Instituto Finlay de Vacunas, Cuba, displays elevated IgG levels, onset of a robust immune response due to the presence of specific long-term memory B-cells, and production of a higher number of neutralizing antibodies in response to antigens. These results ensured that SOBERANA02 could be a potential vaccine candidate for SARS-CoV-2 and paved the way for its clinical trial. Currently, the vaccine is in phase-3 of the clinical trial [61,62].

There has been recent progress in constructing a cervical cancer vaccination using tetanus toxoid. A study on a vaccine developed by the combination of bacterial tetanus toxoid and tumor antigens demonstrated its effectiveness by improving the homing of lymph nodes in the mice model. The efficacy of DC, migration of memory T-cell to spleen, and tumor site were improved by pre-conditioning the mice with tetanus toxoid antigens. The mouse injected with tetanus toxoid, and tumor-cell-based vaccine (GVAX) survived for a longer duration than the mouse injected with Tetanus toxoid or GVAX. Results retrieved from the study confirmed that in Human Papilloma Virus-infected individuals, the combination of tumor antigen and tetanus toxoid induces an anti-tumor effect along with an elevated immune response [63].

Interaction of conventional vaccines and T-Cell/B-Cell for antibody production

T cells and B cells both activate our bodies' immune systems to shield us from numerous pathogenic agents. There has long been debate about their mechanism of action and the relationship with vaccine efficacies. Numerous studies have depicted that the majority of the vaccine-induced immune response is due to antibody production, as illustrated by immunodeficiency, immunological, and passive protection studies. After vaccine administration, the innate immune system in our body detects the antigenic epitopes. The component of the innate immune system comes forward to opsonize the antigen, also different APCs like macrophages or monocytes engulfs the antigen [75-77].

The conventional protein antigens, injected into the body, are taken up by APCs for instance dendritic cells (DCs). The DCs after receiving a sign of threat by the pattern recognition receptors (PRRs) get activated and get passed on to the lymph nodes. Then the MHC-I and MHC-II express processed protein antigens on their surface which further detected by the T cell receptors (TCR) leading to the T cell activation of cytotoxic T lymphocytes. The binding of APCs presenting the antigens, to T cells leads to the immune response activation by forming immune synapses. Immune synapses are complexes forming a bull's eye-type pattern by combining three clusters. The T cell-MHC complexes forming central supramolecular activation cluster (cSMAC), intercellular adhesion molecule-1 (ICAM-1), and lymphocyte

function-associated antigen (LFA1) form the peripheral cluster (pSMAC) and the CD43, CD45 rich distal region (dSMAC). The bull's eye-type pattern is the key to T cell activation [19,22,77,78].

Upon any immune response generation first the B cells and T cells present in the primary lymphoid organ migrate to the secondary lymphoid organs such as lymph nodes, Peyer's patches, spleen, etc. APCs such as macrophages and monocytes, as well as DCs, capture antigen and present it to naïve B and T cells in lymph nodes. The lymph node has 3 regions: an outer region for antigen sampling, a B cell activation zone, and a T cell activation zone. The outer zone contains APCs in enormous amounts which further deliver antigens to B and T cell zones. The B and T cells reside in the specific B and T cell zones which further get activated by APCs expressing processed antigens and differentiate into effector cells. After immunization, the effector cells, for instance, Plasma B cells and Cytotoxic T cells migrate to the injection site [78].

The possible entry portals in the body like mucosal tissues are guarded by naïve CD8+ T cells that differentiate into effector cells. The effector T cells either circulate or resides in tissues and upon antigenic response provide immediate protection. Many precursor cells are present in the T cell-rich zone of lymphoid organs that upon clonal expansion send a signal of antigenic entry and differentiate to T effector cells [79]. When the body encounters a pathogen post-vaccination, the CD8+ memory T cell starts rapid proliferation and the CD8+ effector T cell eliminates the infection-causing cells.

In the lymph node, T cell activation along with the BCR signaling helps in B cell development leading to T cell-dependent B cell development. The developed B cell produces mature high-affinity antibodies. Another event of plasma cell differentiation, antibody, and memory B cell production occur after B cell proliferation. Short-lived plasma cells are formed from naive B cell differentiation after the T cell-B cell interaction, and it further leads to unmutated antibody IgM production. After passing through the germinal centre, naïve B cells either become memory B cells or LLPCs. The LLPCs resides in the bone marrow having the capacity to produce antibodies for a prolonged period [19,22,77].

The process of high-affinity antibody production and maturation is guided by a subtype of T helper cells called follicular Th cells (Tfh). Tfh help in B cell differentiation, and memory B cell generation by producing IL-21. The human naïve and memory cells can be differentiated into antibody-secreting plasma cells by IL-21. The CD40 post ligation on B lymphocytes by CD40 ligands and upon expression on Th activated cells send signals for B cell germinal center response. IL-21 upon CD40 engagement, during T cell-dependent B cell response, helps in plasma cell differentiation by cross-linking and class switch recombination (CSR). The IL-21 co-stimulation induces the expression of BLIMP-1 (B lymphocyte induced maturation protein-1) and the production of a considerable number of IgG from memory B cells without inducing somatic hypermutation [80].

Efforts have been made to develop T cell-inducing vaccines for an exceptionally long time. The first T cell-inducing vaccine developed was BCG. The T cell vaccines are designed in such a way that they would induce CD4+ helper T cells and/or CD8+ cytotoxic effect. Here either the helper T cells will oversee immune response induction, or the CTLs will help in killing the infected cells. Some vaccine candidates for T cell induction against HIV-1 [81], Ebola [82], Malaria [83], Hepatitis-C [84], Influenza [85], Tuberculosis [86], and cancer [87] are still under development.

Role of number of dosages in immunization

Among different determinants of vaccine responses, the role of optimal doses is crucial for antibody production and retention. Currently, the vaccines for COVID-19 in use have a primary dose range of 1–3, depending on the product. In certain cases, the initial dose response is adequate to prevent the necessity for a booster dose. Generally, a booster dose is given to a population which has been administered with a complete standard primary dose series and over time their immunity or clinical protection has decreased below rate. Boosters, restore the fallen effectiveness which was insufficient for the population. In immunocompromised patients and older adults, the standard primary dose series poorly induce the immune response required to fight against the diseases. So, the booster dose that increases its effectiveness seems to be a lifesaver in such cases. Recently for COVID-19, the evidence of waning vaccine effectiveness and circulation of Variant of concerns (VOCs) led to booster administration in a targeted high at-risk population [88]. As a rule, normally an interval of 3 weeks is supposed to be maintained between primary doses as it allows the development of successive antigen-specific primary responses. To induce a higher secondary response by allowing affinity maturation of memory B cells, the interval between primary dose and boosters is decided to be a minimum of 4 months [88].

The role of dosage of antigen, in addition to the interval, is critical in memory B cell responses. A single dose of a few non-live or inactivated vaccines like hepatitis A and Human papillomavirus induce high antibodies which retain for a long time in healthy adults [89]. Primary vaccination series consist of a minimum of two doses given at 3 to 4 week intervals to stimulate consecutive primary B cell and germinal centre responses. Longer intervals between doses seem to enhance the immune response. High antigen doses favor plasma cell induction and lower antigen doses induce immune memory at priming [90]. If the interval between priming and boosting is less than 4–6 months, then the booster response becomes weaker. A higher antigen dose during boosting raises a stronger response by recruiting memory B cells in enormous amounts. Numerous studies involving Inactivated Polio Vaccine (IPV), and Oral Rotavirus vaccine (ORV) demonstrated the positive effect of increasing doses of vaccines. IPV depicted a linear relation between doses administered and antibody generation. Maternal antibody interference causing reduced vaccine efficacy in developing countries is observed in oral rotavirus vaccine. ORV indicate that the increase in doses can overcome the inhibitory effects of maternal (breast milk transferred or transplacental) antibodies [91-93].

The interval between doses and the age of immunization influences the infant immune response generation. The prenatal and postnatal age during immunization determines the early life antibody responses. In accelerated infant immunization series

that comprise doses given at one-month intervals, such as 2,3,4 months or 3,4,5 months, a weaker immune response is elicited. In comparison, doses given at longer intervals, such as 2,4,6 months, can elicit a higher immunological response. However, the vulnerability to infection might reoccur after 12 months of immunization because of the waning of antibody titers which returns to a baseline. In preterm infants, the affinity maturation of vaccine-induced B cells by somatic hypermutation and isotype switching event continues up to one year. But for affinity maturation, needs several months which is why young infants lack high-affinity responses [94].

To maintain a long-term vaccine efficacy persistence of memory B cells is crucial. A study on the smallpox vaccine, on the repeated administration decades after priming showed that the extended memory of live attenuated vaccines can be a result of antigen persistence on the follicular dendritic cells surface. The memory B cells can survive for several decades even without repeated exposure to antigens. Memory B cell undergoing homeostatic polyclonal activation contributes to the persistence of antibody response and renewal of bone marrow plasma cells. According to CDC, the vaccination takes 2 weeks to work against SARS-CoV-2 [95,96].

A comparative study of inactivated COVID-19 vaccine, Varicella vaccine, and Hepatitis-B vaccine demonstrated the relation between the vaccine dose and interval affecting antibody production. For COVID-19 three inactivated vaccine candidates developed by Sinopharm Beijing Institute of Biological Products Co., Ltd., Sinopharm Wuhan Institute of Biological Products Co., Ltd., and Sinovac Life Sciences Co., Ltd. revealed the neutralizing antibody (NAb) rate reached more than 90% at Day 28 after second dose administration following the two-dose schedule of interval 14, 21 or 28 days. The vaccine candidate of Beijing Institute, a post-second dose of two-dose immunization schedule developed NAbs 170, 283, and 218 for intervals 14, 21, and 28 days, respectively. The antibody level after two doses at 14 days intervals was lower than the antibody levels at 21- and 28-days intervals. But between 21- and 28- days interval no notable change in antibody levels was observed. The neutralizing antibody-positive rates at 28 days following the second dose of the two-dose vaccination schedule at intervals of 14 and 21 days were both 98 %, and the neutralizing antibody titer was 121 and 247, respectively, in the Wuhan institute's vaccine candidate. The antibody levels for an interval of 21 days were higher than an interval of 14 days. Sinovac Life Sciences' vaccine candidate demonstrated that the neutralizing antibody-positive rates at 14 and 28 days following the second shot of the two-dose vaccination regimen were 94% and 98%, respectively. The antibody level at an interval of 28 days was higher than 14 days interval. All these clinical trial results suggested that a two-dose immunization schedule at an interval of 14–28 days will provide an effective antibody rate and with an extended interval, improved antibody levels can be seen post full dose vaccination. The two-dose of Varicella vaccine given at intervals of 3 and 6 months showed no significant statistical difference in seropositive rate, in children. The 3 doses of the Hepatitis-B vaccine given at intervals of 5,6 and 8 months between the first and third dose showed no significant statistical seropositive rate, in newborns. Moreover, the studies indicated that different two-dose vaccines for COVID-19 given in the interval of 21 days to 7 months provide an effective immune response. One dose of the inactivated vaccines is insufficient to elicit the immune response necessary to resist the disease, whether a long interval between the first and second dose increases the risk of infection. Therefore, it is necessary to complete the dose regime of a vaccine for an individual to completely immunized against a pathogen [97].

Few vaccine candidates like Pfizer, Moderna, Johnson and Johnson, Sinopharm, and Covaxin for COVID-19 are used as booster doses in different countries. Covaxin developed by Bharat Biotech is now the first vaccine in India to report the immunogenicity and safety results from its booster clinical trial. The NAbs post booster dose administration was heightened by 5 times than the second dose and led to pronounced CD8 and CD4 responses. The booster dose of Covaxin induced robust NAbs against Omicron and delts variants. The test serum sample showed 100% effectiveness against delta and 90% against Omicron [98,99].

A study of the BNT162b2 mRNA vaccine post 4 weeks of first dose administration showed a median 50% FRNT50 (focus reduction neutralization titer) of 102 in a majority of infection naive participants. Against beta and delta variants the NAb level was limited post one dose administration. The alpha variant was not considered in the study, but the previous comparative studies indicated a titer decline up to 3-fold following a peak that was boosted post-second dose. In naive participants, the multiplex ELISA (MSD) measured the anti-S antibodies which were seen to decline first after one dose and boosted after the second dose. The interval extension even showed higher antibody content post-second dose administration [100]. Another mRNA vaccine candidate mRNA-1273, developed by Moderna, showed that from Day 29 the NAb GMT increased post one dose of immunization. Post the second dose, the NAb GMT reached a maximum value of 1733 from Day 43 (14 days after the second dose) and remained heightened through Day 57 [101]. One month after the completion of the primary series immunization schedule, the booster doses of mRNA-1273 were clinically tested. The study used three booster vaccines mRNA-1273.211, mRNA-1273.351, and mRNA-1273, and among them, mRNA-1273.211 showed the highest GMT increase against the VOCs [102].

A protein subunit nanoparticle vaccine candidate Novavax (NVX-CoV2373), containing adjuvanted trimeric SARS-CoV-2 spike glycoproteins, was administered as a two-dose immunization schedule with a 3-week interval to adults <60 years of age. The vaccine reported neutralizing responses and was well tolerated [103].

A meta-analysis of COVID-19 vaccines of different platforms, for instance, mRNA vaccines (mRNA-1273, BNT162b1), adenovirus vector vaccine (ChAdOx1 nCoV-19, non-replicating adenovirus type 5, Sputnik-V), and inactivated virus vaccine (BBIBP-CorV) compared the neutralizing antibody titers and effectiveness of the vaccines. The comparative study on adenovirus vectors Covishield, Adenovirus Type-5, and rAd26 and rAd5 vector-based heterologous Sputnik-V reported that the virus particle of 10^10–10^11 induced NAbs post vaccination against RBDs within 0–28 days. Studies on mRNA vaccines BNT162b1, BNT162b2, and mRNA-1273 reported induction of NAbs post-vaccination within 0–28 days by 25–30 mg dose administration.

This meta-analysis showed that the efficacy of the adenovirus vector collectively was 73% and for mRNA vaccine was 85%. Two subunit vaccines NVX-CoV2373 and MF59-adjuvanted spike glycoprotein-clamp vaccine of Australia reported neutralizing responses. The Australian vaccine reported NAbs in 99% of participants within 57 days of immunization. The nanoparticle-based NVX-CoV2373 vaccine-induced anti-S IgG and NAbs within 28 days post-immunization. The meta-analysis concluded that after 30 days of vaccine administration with both first and second doses the COVID-19 mRNA, adenovirus, and inactivated vaccines generated NAbs against RBDs. However, the overall vaccine efficacy decreased over time for mRNA and adenovirus-based vaccines [39,104-107](See Fig. 3).

Nanocarriers in the advancement of next generation vaccine

Nanocarrier holds enormous potential in the advancement of next-generation vaccines, which has numerous benefits, including improved CD8+ T cell response, improved adjuvant and antigen co-delivery, and the ability to carry nucleic acids [110]. Liposomes, Bilosomes, Poly(lactic-co-glycolic acid) (PLGA), carbon nanoparticles, biomimetic, Gold, Silver, Nano-emulsion, alginate, nanogels, chitosan are a few of the nanocarriers that help in vaccine delivery, and some act as adjuvant as well [111-113]. Some biocompatible nanocarriers, such as Poly (lactic-co-glycolic acid) (PLGA) and Liposomes, are already in clinical use and approved by the Food and Drug Administration (FDA) [110]. The nanocarrier can deliver cytokines and toll-like receptors in addition to nucleic acids. Targeted cell delivery to immune stimulator dendritic cells with increased antigen cross-presentation is also feasible using nanocarriers [113,114].

The shape, size, surface charge, and hydrophobicity of the nanoparticles play a crucial role in cellular uptake, interaction with antigen presenting cells (APCs) and soluble proteins, recognizing hydrophobic moieties, intracellular trafficking, and targeted delivery of the immune stimulators [114]. Nanoparticles are promising immune cell activators and antigen carriers as they can enhance the formation of inflammasome complex and defence gene synthesis. This inflammasome complex further starts the inflammatory reaction for the recruitment of immune cells [110,114]. Nanocarriers are also associated with tumor and nasal vaccine development [16,113]. The use of nanocarriers to induce cellular and humoral immune responses in mucosal immunization against infectious disease is displaying promising results. Nanocarriers are also critical to the delivery of peptide vaccines in advancing anti-tumor vaccines that address immunosuppression [111,112]. Table.2 lists the nanocarriers, their advantages and disadvantages, and the diseases for which they are employed. Fig. 4 shows the diagrammatic representation of nanocarriers with a brief description.

Gold nanoparticles (AuNP) and silver nanoparticles (AgNP) are metal nanoparticles that have gained increasing attention in vaccine development due to their physiochemical properties. These nanoparticles can function as both a vaccination delivery system and an adjuvant. These metal nanoparticles are being studied extensively for their potential therapeutic use in cancer and infectious diseases. By electrostatic, Van der Waals, coordination, and hydrophobic interactions, gold nanoparticles are functionalized with proteins and nucleic acids in direct or indirect methods. Au and Ag nanoparticles can form conjugates and be functionalized in order to stimulate mucosal immunity against a variety of infectious diseases [115-117].

Nanoemulsions are heterogeneous mixes of oil and water stabilized by surfactants. As sophisticated, therapeutic, and protective platforms, oil-in-water nanoemulsions are on emergence. Tuneable surfaces and encapsulation capacity, which improve drug pharmacokinetics, make nanoemulsions intriguing technologies for therapeutic purposes. Oil droplets of nanoemulsion larger than 200 nm are more likely to be phagocytosed than nanoemulsions with around 10 nm oil droplets, which are subject to glomerular filtration. The amount of cytotoxicity is also impacted by particle size; large oil droplets usually result in haemolysis [118]. For cancer immunotherapy, nanoemulsion can be employed as a targeted delivery system. To improve the release of inflammatory cytokines and enhance the immunotherapy using chimeric antigen receptor T cells, nanoemulsion can target the cross-presenting dendritic cell [119]. Moreover, the nanoemulsion can also be employed to encapsulate toll-like receptor (TLR) agonists to target the TLR transmembrane protein to induce potent innate and adaptive immune response [120].

Nanogels are three-dimensional nanoparticles synthesized by the crosslinking of hydrophilic polymeric chain (natural or synthetic) interconnections. When compared to other organic or inorganic nanoparticles employed as delivery methods, nanogels offer substantial benefits due to the inherent characteristics of the polymers used for nanogel fabrication. Nanogels can retain a substantial amount of water and integrate bioactive drugs inside their nanoscale 3D polymer networks, which makes them distinctive from other polymeric nanocarriers in these aspects [121]. There are numerous methods for synthesizing nanogels with specified sizes, shapes, deformation, and surface chemistry. Constrained monomer polymerization with a crosslinking agent is used to synthesize nanogels. Their physicochemical stability is determined by the degree of polymerization, and they are synthesized by chemical or physical crosslinking [122].

Nucleic acid-based vaccine

Nucleic acid-based vaccines are among the most promising prophylactic and immunotherapeutic options due to its humoral and adaptive immunity deriving potential and specificity to target various tumour antin for a robust immune response against cancerous cells [123]. Fig. 1 shows the vaccines and mechanism of immune response activation. Nucleic acid delivery is mediated by nanocarriers such as lipid, inorganic, natural polymers, and peptide-based nanoparticle systems [19]. The most clinically advanced nanoplatforms for RNA delivery is LNPs. The primary emphasis of LNP research was on cationic lipids, which can electrostatically absorb polyanionic RNAs. Because of their toxicity at the site of delivery, the usage of cationic liposomes is constrained. Many laboratories and pharmaceutical companies have begun to use ionizable lipids, which are positively charged only at acidic pH. Inside the cell, the positive charge on the ionizable lipids favors the complex formation with the nucleic acid [124,125]. LNPs exhibit significant kinetic and rigid morpholog-

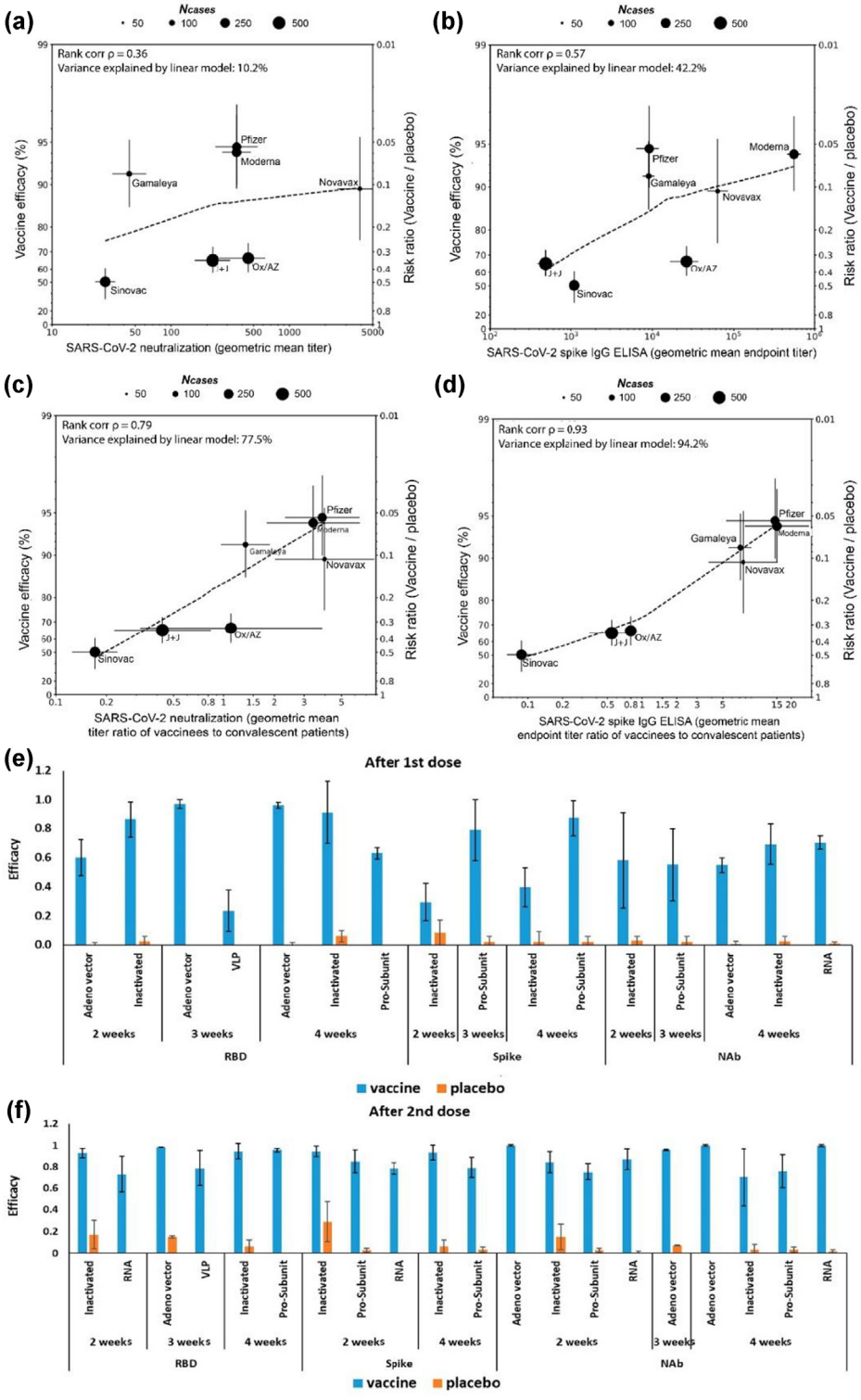


FIGURE 3

Graphical representation of the correlation between doses, antibody responses, and efficacy rate of different COVID-19 vaccine platforms. (a) Demonstrates the correlation of neutralizing antibody responses using geometric mean titer (GMT) value without Human convalescent serum (HCS) consideration. (b) The ELISA assay ratio of spike IgG without considering the HCS. (c) The correlation of neutralizing antibody responses using GMT value with HCS. (d) The ELISA assay ratio using geometric mean endpoint titer ratio of vaccines considering HCS. Here, the X-axis represents the ratio of the peak GMT titer for (a) & (c) and ELISA titer for (b) & (d) post-vaccination at 7–28 days. The Y-axis represents the estimated log risk ratio reported on the vaccine efficacy scale (Error bars imply 95% Confidence Intervals). Figure (a-d) is adapted and modified from [108]. (e) & (f) demonstrate the correlation between the efficacy of 7 different COVID-19 vaccine platforms after administering the first and second doses, respectively in both vaccine and placebo groups. Figure (e) & (f) are adapted and modified from [109].

TABLE 1

Emergency Use Authorized (EUA) vaccine candidates for COVID-19. IM, Intramuscular.

Candidate	Platform	Developer	Route of administration	Number of doses	Phase	Efficacy	Reference
Covishield (AZD1222)	Viral vector vaccines (non-Replicating)	AstraZeneca+ University of Oxford	IM	1–2	4	B1117 variant-61.7% Other variants-77.3%	[42]
BNT162b2	RNA vaccine	Pfizer/BioNTech + Fosun Pharma	IM	2	4	95%	[64]
mRNA-1273.351	RNA vaccine	Moderna + National Institute of Allergy and Infectious Diseases	IM	2–3	4	94.1%	[65]
BBIBP-Cor-V	Inactivated vaccine	Sinopharm + China National Biotec Group Co+ Beijing Institute of Biological Products	IM	2	4	78.1% overall	[66]
Sputnik-V	Viral vector vaccines (non-Replicating)	Gamaleya Research Institute; Health Ministry of the Russian Federation	IM	2	3	91.6%	[67]
Ad26.CoV2.S	Viral vector vaccines (non-Replicating)	Janssen Pharmaceutical Johnson & Johnson	IM	1–2	4	66.9% symptomatic	[68]
CoronaVac	Inactivated vaccine	Sinovac Research and Development Co.,Ltd	IM	2	4	50–84%	[69]
Covaxin (BBV152)	Inactivated vaccine	Bharat Biotech International Limited	IM	2	3	Overall-77.8% Delta variant 65.2%	[70]
Ad5-nCoV (Convidecia)	Viral vector vaccines (non-Replicating)	CanSino Biological Inc./Beijing Institute of Biotechnology	IM	1	4	65.7%	[71]
Inactivated SARS-CoV-2 vaccine	Inactivated vaccine	Sinopharm + China National Biotec Group Co+ Wuhan Institute of Biological Products	IM	2	4	72.8%	[72]
EpiVacCorona	Subunit vaccine	Federal Budgetary Research Institute State Research Center of Virology and Biotechnology "Vector"	IM	2	3	Not yet published	
CIGB-66 (Abdala)	Protein subunit vaccine	Center for Genetic Engineering and Biotechnology, Cuba	IM	3	3	92.28%	[73]
FINLAY-FR-2 (Soberana 02)	Conjugate vaccine	Finlay institute, Cuba	IM	2	3	71%	[74]
QazVac	Inactivated vaccine	Research Institute for Biological Safety Problems, Kazakhstan	IM	2	3	Not yet published	
Sinopharm/ Hayat-Vax	Inactivated vaccine	Sinopharm China + Group 42 & Julphar Abu Dhabi	IM	2	3	86%	[69]
ZF2001 (RBD-Dimer)	Protein subunit	Anhui Zhifei Longcom Biologic Pharmacy Co., Ltd. + Institute of Microbiology of Chinese Academy of Sciences	IM	2–3	3	Not yet published	

ical stability and more homogenous LNPs can be obtained by the large-scale commercial fabrication method. Phospholipids, ionizable lipids, cationic lipids, cholesterol, and PEG lipids constitute the lipid-based nanoparticles [126]. In the protection of nucleic acid from nuclease degradation ionizable lipid plays a key role. To entrap siRNA approximately 30–40 mol% of helper lipids such as phospholipids and cholesterol are required [127]. This helper lipid promotes membrane fusion and ensures the stability of formulation [128]. PEG lipid content can improve the circulatory half-life and stability due to the conjugation of hydrophilic PEG polymer and hydrophobic lipid anchor which lead to the prevention of non-specific protein adsorption and re-establishing the specific binding [127]. At physiological pH, ionizable LNPs have a near-neutral charge, but at low pH, the amine groups on ionizable lipids become protonated and positively charged, facilitating assembly with phosphate groups (negatively

TABLE 2

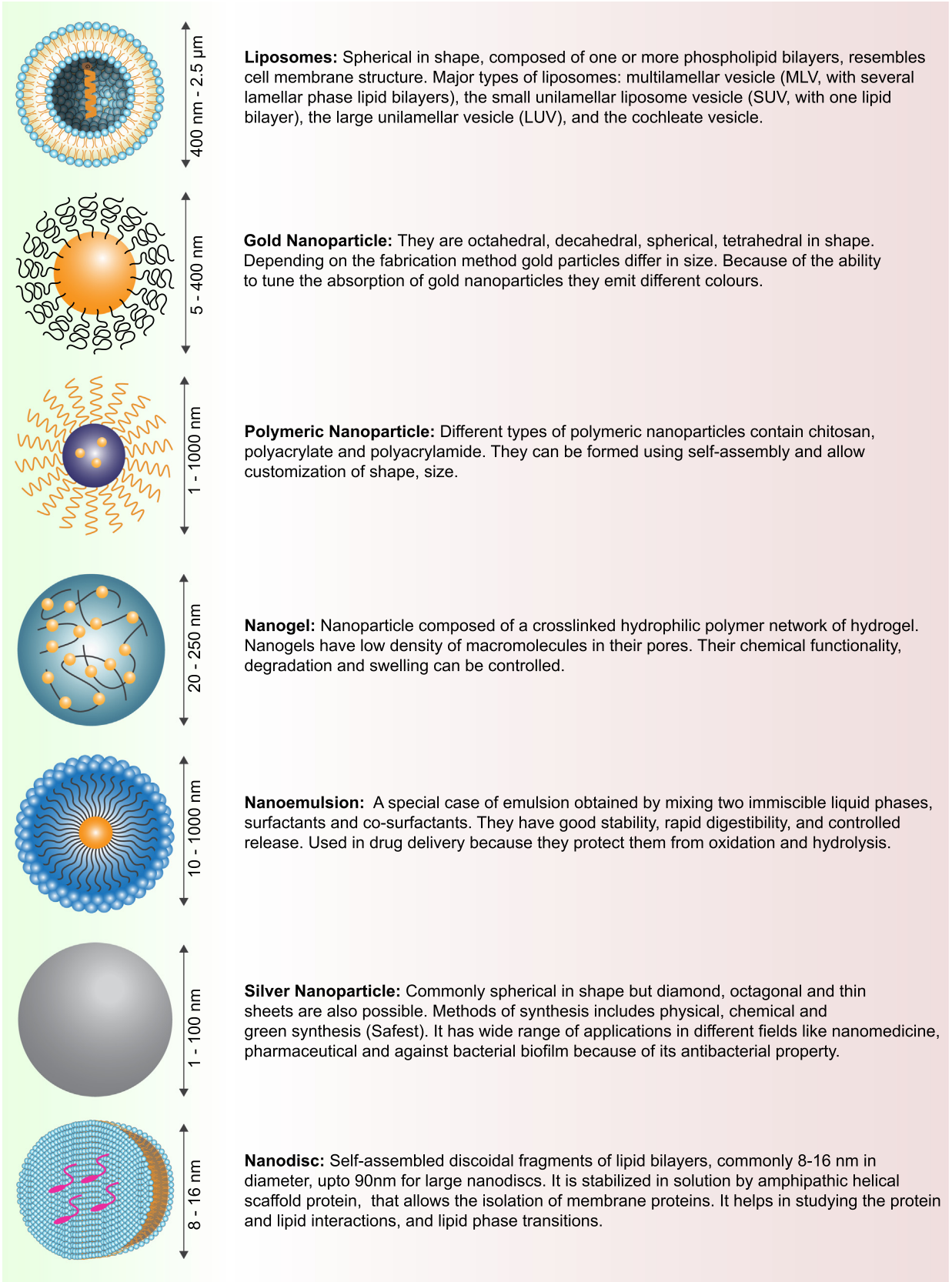
Different nanocarriers, their advantages and disadvantages, and the diseases for which they are employed. Modified from reference [111].

Nanocarriers	Carries	Advantage	Disadvantage	Disease
PLGA	• Antibiotics • Anti-inflammatory drugs • Proteins/peptides • Nucleic acids	• US Food and Drug Administration Approved • Biodegradable, biocompatible, and no safety concern • Can be formulated to the nanoparticle or microparticle • Various antigens with full antigenicity can be loaded within PLGA or PLGA-based conveyor • Recognizable by professional APCs	• Antigen Instability during encapsulation, drying, and storage	Influenza A Dengue Hepatitis B Hepatitis C Anthrax
Liposomes	• Anti-cancer drug • Nucleic acids • Water-soluble proteins	• Biodegradable, biocompatible, non-toxic, and non-immunogenic • No Safety concerns • Resemblance to biomembranes • Protect encapsulated hydrophilic, hydrophobic, and amphipathic antigens • Can be formulated for nanoparticles or microparticles • Can be administered through various routes improve transfection • Controlled release	• Low solubility • Short half-life • High cost • The formulation is highly dependent on antigen charge and size • Instability • poor permeability • Special storage requirement	SARS-CoV-2 SARS-CoV Hepatitis C Hepatitis E
Lipid nanoparticle	• Anti-cancer drug • Small molecule drug • Nucleic acid • Protein	• Biocompatible and biodegradable in nature • Good stability during storage period • Easy and scalable production process • Short onset action and longer duration time • High encapsulation efficiency • Good stability • Sustained and control release. • Can be administered through various routes • Lower cytotoxicity	• Lost of high amount of drug in topical delivery • Particle size growth during storage time • Polymorphic transition • Drug expulsion	Cancer COVID-19 Parkinson's Bacterial skin infection
Bilosomes	• Drugs for cutaneous delivery and oral delivery • Antigen • Phytomedicine	• Self-adjuvant properties • No special storage requirement • High antigen encapsulation • Protect antigens in the GI tract, rapid and efficient uptake by M cells • Induce mucosal immunity at the site and other distant mucosal sites	• Unstable in GI environment	Hepatitis B Diphtheria Toxoids Influenza
Gold Nanoparticles	• Large biomolecule • 'Free' drug • Genes	• Readily internalized by macrophages and dendritic cells • A wide range of molecules, (adjuvants and antigens) can be conjugated • Large scale production is possible	• Could be associated with organ toxicity as accumulates in liver and spleen for a longer period	Tetanus toxoid
Silver Nanoparticles	• Anti-cancer drugs • Antibiotics • Gene • Antisense oligonucleotides	• Has rigid structure • Low-cost production • Can be used as an anti-bacterial and larvicidal agent • Non-toxic	• Oral toxicity • Immunotoxicity • Neurotoxicity • Environmental toxicity	Cancer
Chitosan	• Biomacromolecules • Anti-cancer drugs	• Non-toxic, biodegradable, biocompatible, and has bio-adhesion ability	• Insoluble at physiological pH in the water • Easy degradation in acidic media such as the GI tract • Irregular distributions	Tuberculosis New castle disease
Alginate	• Drugs (act as excipient)	• Low toxicity, biocompatibility, biodegradability • U.S. Food and Drug Administration approved • Stable in gastric fluid	• Incompatible with heavy metals • Cannot be fully eliminated from our body • Non-degradable in mammal	Tuberculosis Anti-tumor chemotherapy Cancer
ISCOM	• Antigen • Vaccine	• Small amounts of encapsulated antigens are immunogenic • Induce humoral and cellular immune responses • Highly stable	• The incorporation of many antigens into the structure is difficult • Not very stable in the gut	Herpes simplex virus type 2 (HSV-2 Influenza

(continued on next page)

TABLE 2 (*CONTINUED*)

Nanocarriers	Carries	Advantage	Disadvantage	Disease
			• Difficult to manufacture • Strong pain at the injection site • Strong toxic reactions	Diarrhea
Nanoemulsion	• Active pharmaceutical ingredients	• Micro-domain of different polarities within the same single-phase solution can facilitate solubility of hydrophilic or lipophilic peptides. • Can be designed in the form of oral dosage.	• The interplay between nanoemulsion, peptides, and physiological conditions in the intestine is unclear. • Bio-relevance must receive more attention	Tuberculosis Anti-tumor therapy
Nanogel	• siRNAs • Oligonucleotide • Chemotherapeutic drugs	• The formation by covalent cross-linking reactions induces stability in a complex and unkind environment, preventing the leakage of the encapsulated drug. • High drug loading capacity.	• Traditional covalent crosslinking agents cause unwanted toxic effects and damage the entrapped peptides.	Cancer Pneumococcal infections
γ-PGA	• Antimicrobial drugs • Anti-cancer drugs • RNA • DNA • Insulin	• Low toxicity • Biocompatible and biodegradable • Induces both cellular and humoral immune response • Aduvant	• Hardly soluble in organic solvents • Easily degraded under acidic, alkaline, and high-temperature conditions	Influenza HIV-1 Japanese encephalitis Diabetes Cancer
Graphene oxide	• Chemotherapeutic drug • Gene	• High aspect ratio • Flexible surface modification • Biocompatible and nonimmunogenic	• After high dose administration retention in body can be a possible issue. • At the moment, it is unclear if the substance will remain in the lungs indefinitely.	Influenza Cancer
Carbon nanotube	• Antibodies • Proteins • DNA	• Unique infrared light-responsive properties • Delivery vehicles for antigens and adjuvants	• Potential long-term toxicity and • Limited biodegradability	COVID-19
Carbon Quantum dots	• Anticancer drug • Neurodegenerative • Antineoplastic drugs	• Highly versatile in size, structure, and geometry	• Potential long-term toxicity and • Limited biodegradability	COVID-19 Cancer



Liposomes: Spherical in shape, composed of one or more phospholipid bilayers, resembles cell membrane structure. Major types of liposomes: multilamellar vesicle (MLV, with several lamellar phase lipid bilayers), the small unilamellar liposome vesicle (SUV, with one lipid bilayer), the large unilamellar vesicle (LUV), and the cochleate vesicle.

Gold Nanoparticle: They are octahedral, decahedral, spherical, tetrahedral in shape. Depending on the fabrication method gold particles differ in size. Because of the ability to tune the absorption of gold nanoparticles they emit different colours.

Polymeric Nanoparticle: Different types of polymeric nanoparticles contain chitosan, polyacrylate and polyacrylamide. They can be formed using self-assembly and allow customization of shape, size.

Nanogel: Nanoparticle composed of a crosslinked hydrophilic polymer network of hydrogel. Nanogels have low density of macromolecules in their pores. Their chemical functionality, degradation and swelling can be controlled.

Nanoemulsion: A special case of emulsion obtained by mixing two immiscible liquid phases, surfactants and co-surfactants. They have good stability, rapid digestibility, and controlled release. Used in drug delivery because they protect them from oxidation and hydrolysis.

Silver Nanoparticle: Commonly spherical in shape but diamond, octagonal and thin sheets are also possible. Methods of synthesis includes physical, chemical and green synthesis (Safest). It has wide range of applications in different fields like nanomedicine, pharmaceutical and against bacterial biofilm because of its antibacterial property.

Nanodisc: Self-assembled discoidal fragments of lipid bilayers, commonly 8-16 nm in diameter, upto 90nm for large nanodiscs. It is stabilized in solution by amphipathic helical scaffold protein, that allows the isolation of membrane proteins. It helps in studying the protein and lipid interactions, and lipid phase transitions.

FIGURE 4

Brief description of the nanoparticles that are used as carriers for nucleic acids and peptides and functionalization with targeted molecules to induce a potent immune response.

charged) on nucleic acids. To facilitate therapeutic delivery, the pH can be changed after complex formation to a neutral or physiological pH. Ionizable LNPs can extravasate from the circulation to target tissues after in vivo injection [129].

In metal nanoparticles, the nanoconjugate is facilitated by the electrostatic interaction of positively charged nanoparticles and negatively charged nucleic acid. The metal nanoparticle e.g., the gold nanoparticle can be functionalized with polymers and thiol ligand containing the mannose-mimicking shikimoyl to synthesize a positively charged metal nanoparticle [130]. Through thiol moieties, nucleic acid strands are covalently bonded to the gold nanoparticle cores, which are generally 13–15 nm in size [131]. Anionic nucleic acids and polycations can be alternately layered on the surface of gold nanoparticles to coat them, and targeting ligands can also be added to enable targeted interactions and adsorption of nanoparticles to cell surface receptors [132-134].

Another class of materials that are appealing for RNA transport is cationic polymers due to their chemical variability, accessibility in surface modification, and synthetic reproducibility. Polyethylenimine is the widely investigated polycation among the numerous polymers because of its comparatively high transfection efficiency. However, significant cytotoxicity prevents its extensive use, particularly for the branched, high-molecular-weight polyethylenimine. Alternative polymeric materials with reduced toxicity include chitosan, a naturally occurring cationic polysaccharide, and polyamidoamine, a dendrimer constructed from methyl acrylate and ethylenediamine [129]. Cationic polymers in polyplexes interact electrostatically with nucleic acids to bind and condense them into small, compact structures [135]. The incorporation of covalent cross-linkers into the particle core or the introduction of hydrophobic components to facilitate particle synthesis through hydrophobic aggregation can both improve the packaging stability of polyplexes [129,136].

DNA vaccine

In the field of next-generation vaccines, immunization through DNA vaccine is quite remarkable. Chitosan, poly (lactic acid) (PLA), poly (glutamic acid) (PGA), and poly (lactic-co-glycolic acid) (PLGA) are the polymer nanoparticles that are primarily used for the delivery of DNA molecules. These nanocarriers have the property of controlled release, the capability to enhance immune response, along with a safety profile. Moreover, lipid nanoparticles, hybrid polymer-based nanoparticles, virus-like particles (VLPs), and protein-based nanoparticles are also employed to deliver the DNA vaccine efficiently. Inorganic nanoparticles, for instance, gold, silver, and ferric nanoparticles provide greater biocompatibility and chemical stability with a well-defined structure. Gold (Au) nanoparticle is applicable for both delivery and as an adjuvant; hence it plays a valuable role in stimulating the innate and adaptive immune responses [137]. The physical method including Electroporation, particle-mediated epidermal delivery (PMED) gene gun, and non-viral gene delivery approaches are considered for the plasmid DNA transport [138,139].

The first application of the DNA vaccine was announced in the 1990s. It injected RNA or DNA molecules into mouse skeletal muscle, producing chloramphenicol acetyltransferase, luciferase, and beta-galactosidase. The candidate gene was identified *in vivo* for up to two months after infection [140,141]. DNA vaccines can effectively elicit both humoral and cell-mediated immunity after delivering the vector DNA plasmid to the host cell consisting of the gene fragment encoding the antigen that can activate the immune response **(**Fig. 5**)** [142,143]. DNA vaccine for influenza was in development since the 90s and got success in the trial of the murine model but in larger animal unsatisfactory result was reported. Throughout the years, the incorporation of gene delivery techniques, vector expressions, vaccine adjuvant, and antigen designing promoted the DNA vaccine platform [142].

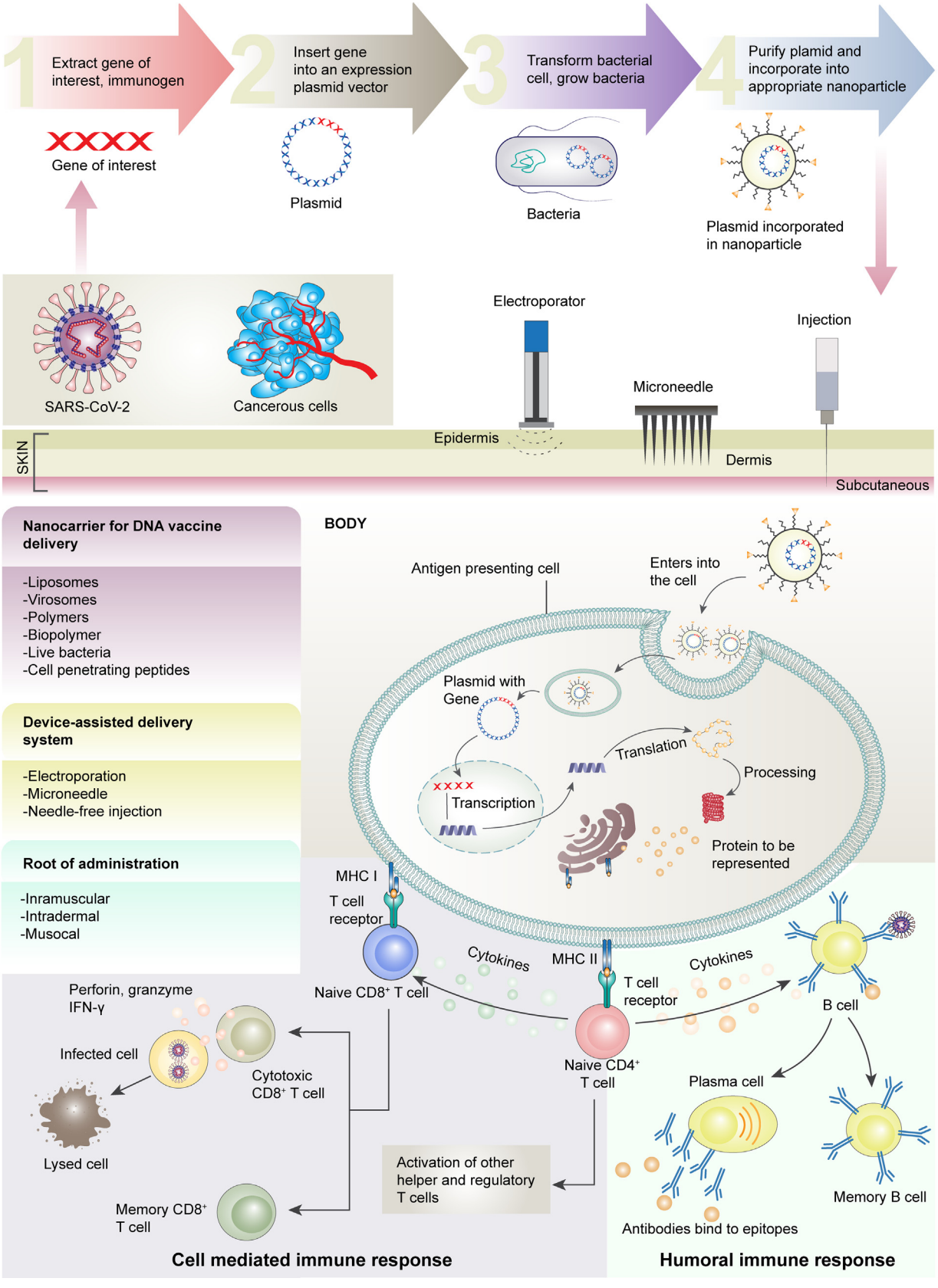
Effective DNA vaccines are licensed for veterinary application only. For the time being, there is no fully licensed vaccine for human clinical except for benefits due to the poor immunization effect [143-145]. However, one plasmid DNA vaccine recently got approved for EUA. It was reported that the DNA vaccine elicited both forms of adaptive immunity along with reducing median viral load in the nasal mucosa and bronchoalveolar lavage in COVID-19 infected rhesus macaques [146]. Cadila Healthcare Limited, an Indian firm has developed a 3-dose plasmid DNA vaccine (ZyCoV-D) against COVID-19. ZyCoV-D displays great tolerability, safety, and robust immunogenicity with both humoral and cell-mediated responses. [147]. ZyCoV-D has entered phase 3 of the clinical trial (CTRI/2021/01/030416) [148] and recently got approved for Emergency Use Authorization (EUA) by the Drug Controller General of India (DCGI). After approval, India has become the first country to develop a licensed plasmid DNA vaccine in the world for human application. Moreover, this vaccine is the first to consider adolescents for clinical trials and employs needle-free administration for COVID-19 vaccination [149]. The interim report shows that ZyCoV-D has primary efficacy of 66.6%, is stable at 25 °C for at least 3 months which may ease the transportation process [150]. However, for COVID-19, more than twenty DNA-based vaccines are in clinical and pre-clinical trials that could be potential candidate in near future [17].

DNA vaccines comparatively provide additional advantages over conventional vaccines by inducing a broader immune response, higher scalability, formulation of multiple antigens

FIGURE 5

DNA vaccine and elicitation of a humoral and adaptive immune response. Gene of interest from SARS-CoV-2 (mainly the spike protein sequence) and tumor or cancer cell is extracted. Insert the gene of interest to an expression plasmid to multiply the copy number in bacteria. Then the gene of interest is incorporated into the appropriate carrier to deliver the plasmid into the body where the antigen-presenting cell takes up the incorporated plasmid through the process of endocytosis. The gene of interest gets transcribed inside the nucleus to form mRNA then translation occurs at the cytoplasm to produce an expressed protein (protein of interest to be represented on the APC). Both humoral and cell-mediated immunity are induced by MHC-I and MHC-II molecules presenting the expressed protein. MHC-I: Major histone compatibility class 1; MHC-II: Major histone compatibility class II; IFN-γ: Interferon-gamma; APC: Antigen-presenting cell.

in one vector in a single vaccine, low-cost production for developing countries, and higher stability for storage. Storage and cold chain distribution in tropical countries is a great hurdle in the vaccinology field. Cold storage is necessary for live vaccine protection and content preservation to ensure vaccine survival. On the contrary, the DNA vaccine requires less refrigeration which



may benefits the distribution of vaccines in the hotspot areas [140].

Besides infectious diseases, DNA vaccines can also be incorporated with cancer immunotherapy. Tondini et al. [151] worked on the cooperation of vaccine-based blockade immunotherapy that synergizes poly-neoantigen DNA vaccine with PD-1 blockade, and they observed the induction of CTLs and T-helper cells cellular response in the mice. This study also demonstrated that, for complete eradication of tumors, multiple neoepitopes can be integrated into a single DNA vaccine formulation. In cancer vaccine progress, controlled release of antigen with robust stimulation of tumor-specific T-cell response is challenging that must be resolved. Recently, Liu et al. [152] have developed a programmable DNA nano-device-based vaccine that can control immunogenic response while working on mice. Besides, it can load multiple adjuvant and antigens to induce the tumor-specific Cytotoxic T-Lymphocyte (CTL) response that can suppress and regress the tumor. Further stated that DNA co-delivery platform with therapeutic vaccines has the potential to prevent the recurrence and metastasis of the tumor. However, for further analysis, a clinical trial is needed.

RNA vaccines

The mRNA is an intermediary step between protein-encoding DNA translation and protein production in the cytoplasm by ribosomes. Two forms of RNA vaccine platform are currently studied, First, Conventional mRNA vaccine: unmodified and/or modified that are chemically and sequence optimized, and; Second, Self-amplifying mRNA (SaRNA) vaccine: replicative and targeted antigen encoded virally derived [15,153]. Only the antigen of interest and its 5′ & 3′ Untranslated region is encoded by conventional mRNA vaccines. Whereas SaRNA vaccines have the potential to amplify RNA intracellularly which leads to better protein expression as it also encodes the replication machinery of pathogens with the antigen of interest **(**Fig. 6**)** [8]. Both forms of mRNA vaccines have already been introduced to several infectious diseases such as Rabies [154], Influenza [155], Zika [155], Ebola [156], and now COVID-19 [156]. mRNA vaccine has several advantages over the conventional inactivated, and live vaccines in safety efficacy and deployment of inexpensive large scale, and scalable manufacturing [8,18]. mRNA vaccines also differ from DNA vaccines in the site of action. DNA vaccines need electroporation to cross the nuclear membrane, whereas RNA vaccines act at the cytoplasm where protein translation occurs. For that reason, the RNA vaccine can be administered with a regular needle injection [15,18].

At least 18 mRNA and self-amplifying mRNA vaccine candidates are in clinical development for COVID-19 [17]. Moderna's mRNA-1273 and Pfizer's BNT162b2 are lipid nanoparticle-based vaccines, approved for Emergency Authorized Use (EUA) that lead the mRNA platform in immunizing the world and speeding the vaccination process. mRNA-1273 immune response in adolescents was evaluated as safe and similar to younger adults [157]. Both the vaccines show robust immune responses and the highest efficacy in their clinical trials [64,65]. The efficacy of EUA candidates is mentioned in Table 1**.**

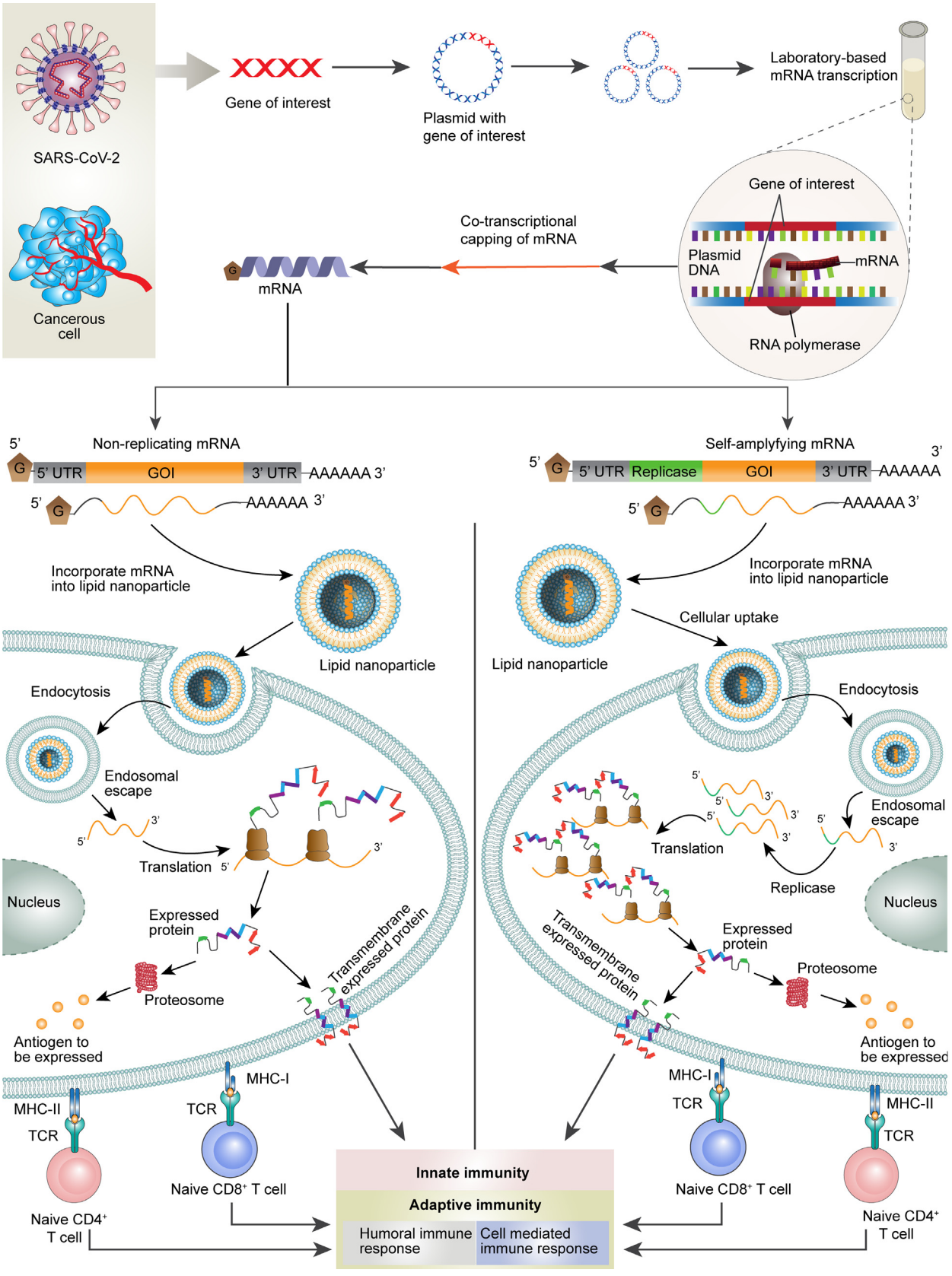
The formulation of both mRNA and SiRNA employed lipid nanoparticles for encapsulation [64,65,158-160]. Self-amplifying mRNA has become an important approach in developing future vaccines as manufacturers reported robust immunogenicity against COVID-19 in their clinical trials [158,161]. The anti-SARS-CoV-2 antibody can induce antibody-dependent enhancement (ADE) and can worsen the symptoms according to several studies. The evidence of ADE in several animal models for instance in macaques after the administration of SARS-CoV-2 protein-expressing Ankara viral vector causes severe lung infections compared to the unvaccinated macaques, raises safety concerns. Clinical evidence for an ADE role in human COVID-19 is still unclear. To minimize the possibility of ADE from immunotherapies, high dosages of robust neutralizing antibodies are introduced or delivered, rather than lesser concentrations of non-neutralizing antibodies, which are more prone to causing ADE [162]. McKay et al. [163] introduced self-amplifying mRNA encoded in lipid nanoparticles that codes for the SARS-CoV-2 protein i.e., the antigen of interest, on mice. High magnitude of IgG neutralizing antibody titter with induction of T helper type 1 (Th1) biased immunization response and no risk of antibody-dependent enhancement was observed.

One of the major challenges in self-amplifying RNA vaccines is the difficulties in translation in the dendritic cells and the RNase sensitivity. However, for effective translation polyplex formulation and fine-tuning the polyplex structure can be the option to consider. Besides, the molecular structure of polyethyleneimine, weight-to-weight ratio, and cell-penetrating peptides are other factors that can enhance the translation process of SaRNA in the dendritic cell. The issue remains challenging for replicating RNA, only specific genes get translated among the multiple genes [164]. Perche et al. [165] in their study on mice encapsulated the RNA into the lipopolyplexes using three corresponding parts, an anionic liposome, cationic polymer, and RNA. This formulation delivered the conventional and replicative RNA to the dendritic cell inside the cell that induced the adaptive immune response successfully in the mice cell. Further stated that the neutral lipopolyplex can be the universal delivery formulation for RNA vaccine delivery.

Another biggest hurdle for vaccine development is the storage and distribution of vaccines. Without sufficient stability in the vaccine structure, it is quite challenging to distribute the vaccine

FIGURE 6

RNA vaccine and elicitation of the humoral and adaptive immune response. Gene of interest of pathogen (e.g., SARS-CoV-2 spike protein) and tumor and cancerous cell antigen is identified and extracted. Antigen encoding nucleic acid is introduced into the plasmid and laboratory-based transcription produces a capped mRNA (non-replication or self-amplifying). mRNA is incorporated into lipid nanoparticles leading to cellular uptake through endocytosis. Endosomal escape of lipid nanoparticles in cytoplasm and release of mRNA. Non-replicating mRNA is translated however in SiRNA due to the presence of the replicase gene it first replicates its mRNA into multiple mRNAs and then gets translated into proteins (antigen of interest). Some proteins express themselves and others are chopped off by proteosome, then antigen-presenting two primary classes of major histocompatibility complex molecules such as MHC-I and MHC-II present the antigen of interest on the cell surface, recognized by immune cells.


SARS-CoV-2
Cancerous cell
Gene of interest
Plasmid with gene of interest
Laboratory-based mRNA transcription
Gene of interest
Plasmid DNA
mRNA
RNA polymerase
Co-transcriptional capping of mRNA
mRNA
Non-replicating mRNA
5' UTR
GOI
3' UTR
AAAAAA 3'
Self-amplyfying mRNA
Replicase
Incorporate mRNA into lipid nanoparticle
Lipid nanoparticle
Cellular uptake
Endocytosis
Endosomal escape
Translation
Replicase
Nucleus
Expressed protein
Transmembrane expressed protein
Proteosome
Antigen to be expressed
MHC-I
MHC-II
TCR
Naive CD8⁺ T cell
Naive CD4⁺ T cell
Innate immunity
Adaptive immunity
Humoral immune response
Cell mediated immune response

in a cold chain procedure. COVID-19 mRNA encoded in lipid nanoparticles needs ultra-low temperature for storage which may slow down the process of distribution. Schoenmaker et al. [166] did a comprehensive survey on the structure and stability of lipid nanoparticles encoding the mRNA of SARS-CoV-2. They concluded that the instability of the mRNA vaccine is due to the water interaction with the mRNA encoded into the lipid nanoparticles. He also suggested that reducing the water exposure to the mRNA would be a promising step towards mRNA vaccine stability. Further suggested that the use of different promising techniques such as drying and lyophilization could help in the stabilization of RNA vaccines. Zhang et. al [167] developed a thermostable mRNA vaccine for COVID-19, antigen encapsulated in lipid nanoparticles that can be stored at room temperature for at least one week. The result was promising with SARS-CoV-2 specific Th 1 biased cellular immune response. Currently, the thermostable LNP based mRNA vaccine is in phase III of a clinical trial (NCT04847102).

For cancer immunotherapy, the mRNA vaccine is a promising platform for therapeutic immunization. More than twenty vaccines that exhibit promising outcomes have entered the clinical trial for solid tumor treatments. However, several limitations remain associated with mRNA vaccines such as the limited innate immunogenic response, the delivery, and instability. To improve the innate immunogenicity, strategies like modification of poly (A) tail, nucleosides, five prime caps (5′cap), optimization of untranslated regions (UTRs), codon optimization, and utilization of type 1 interferons are being done. Moreover, to enhance the delivery efficiency of ionizable lipid nanoparticle delivery system, polymer-based delivery systems (cationic polymer: Polyethylenimine; dendrimer: Polyamidoamine; biodegradable polymers) and peptide-based delivery systems are used [123]. Recently, the Institutional Review Board (IRB) committee of NIH and FDA approved a phase I/II (NCT03480152) mRNA vaccine for safety and immunogenicity in the clinical trial of 4 individuals that induces a neoantigen-specific T-cell immune response in the gastrointestinal cancer patients [168]. Another liposomal mRNA vaccine [169] is in phase 1 of a clinical trial that targets the prevalent melanoma non-mutated, tumor-associated antigen that drives immunity to checkpoint-inhibitor to treat melanoma. This melanoma vaccine FixVac (BNT111) illustrates strong clinical responses by inducing $CD4^+$ and $CD8^+$ T cell responses.

Safety and immunogenic concerns have been raised about the live attenuated TC-83 vaccine used for the Venezuelan equine encephalitic virus (VEEV), including reactogenicity and vaccine inability to seroconvert. Samsa *et al*. [170] by conveying genetically engineered VEEV genomes with synthetic cationic nano-emulsion developed a novel VEE vaccine using the concept of self-replicating mRNA. The nanoemulsions have been optimized by the vaccine industry for acting as an adjuvant. The advantage of using a cationic nano-emulsion (CNE) for VEEV vaccine development is that it may be manufactured independently from RNA, and its enhanced delivery leads to an increase in the efficacy of the vaccine [171]. The vaccine can be stored and utilized efficiently in the event of a pandemic or the outbreak of an epidemic [172]. The vaccinated mice demonstrated 100% protection from VEEV and induced similar immunogenicity to the TC-83 live attenuated vaccine. Further, they stated that this concept is proof to speed up the vaccine development of other alphaviruses including the chikungunya virus. In another study, Szurgot et al. [173] employed infectious RNA to develop a novel platform for vaccine development against the chikungunya virus. This novel platform elicited protective immunity against the virus after a single immunization in mice. They believed that the infectious RNA platform is also applicable to other positive-stranded viruses. Apart from VEEV, a few pre-clinical studies conducted on animal models showed that the CNE-formulated self-amplifying RNA vaccines induced immune response against HIV, malaria, influenza virus, and cytomegalovirus [171,172,174–176].

As the instance of COVID-19 has shown, mRNA vaccines can be rapidly produced relatively at a lower cost because, as soon as the genome sequence of the targeted antigen is identified, the production process starts immediately. Pandemic threats like COVID-19 and other infectious diseases need a rapid response in vaccine production and manufacturing program. Self-amplifying RNA vaccines have the potential to overcome these challenges [177]. New studies are continually being conducted in the field of RNA vaccinology, to potentially enhance in vivo pharmacokinetics, improve in vitro transcription, and delivery formulations, and optimize adjuvant [153].

Subunit vaccine

Subunit vaccine candidates comprise minimum structural components of the virus that can elicit protective immune responses in the host. Subunit vaccines are administered with molecular adjuvants for better immune responses. In addition to providing various benefits intrinsic to each nanocarrier platform, subunit vaccines can consist of viral proteins incorporated in synthetic nanomaterials, protein cages, and Virus-like particles, which act as adjuvants and may also act as delivery vehicles [25]. Fig. 7 represents the identification and immune response mechanism of the subunit vaccine. For COVID-19 more than 57 candidates of protein subunit and virus-like particle vaccine platforms are in the clinical trial and more than 19 protein subunit vaccines are in phase 3 of the clinical trial [17]. Full-length spike glycoprotein and Receptor binding domain (RBD) is more prevalent among the vaccine candidates. Trimer-Tag technology is used to develop an S-Trimer-based subunit vaccine that displays resemblance to the SARS-CoV-2 S1/S2 subunit structure and function. It induces a protective immune response in presence of adjuvant against the virus in nonhuman primates. A vaccine NVX-CoV2373 developed by Novavax leads the protein subunit vaccine race with an efficacy of 86.3% against the alpha variant and 94.6% against the non-alpha variant of SARS-CoV-2 [178]. Another firm, SK Bioscience Co. Ltd, is conducting a phase I/II study using a SARS-CoV-2 receptor-binding domain (RBD) nanoparticle scaffold termed RBD-NP with different adjuvant that produced substantial protective response against the virus [179]. Studies are currently being conducted to determine if the RBD or spike protein promotes superior immunogenicity. However, recombinant S and S1 protein were reported to be superior in triggering stronger immunogenicity in mice.[180,181].

Multi-epitope subunit vaccine (MESV): MESV is a novel platform that rapidly gaining attention in the field of vac-

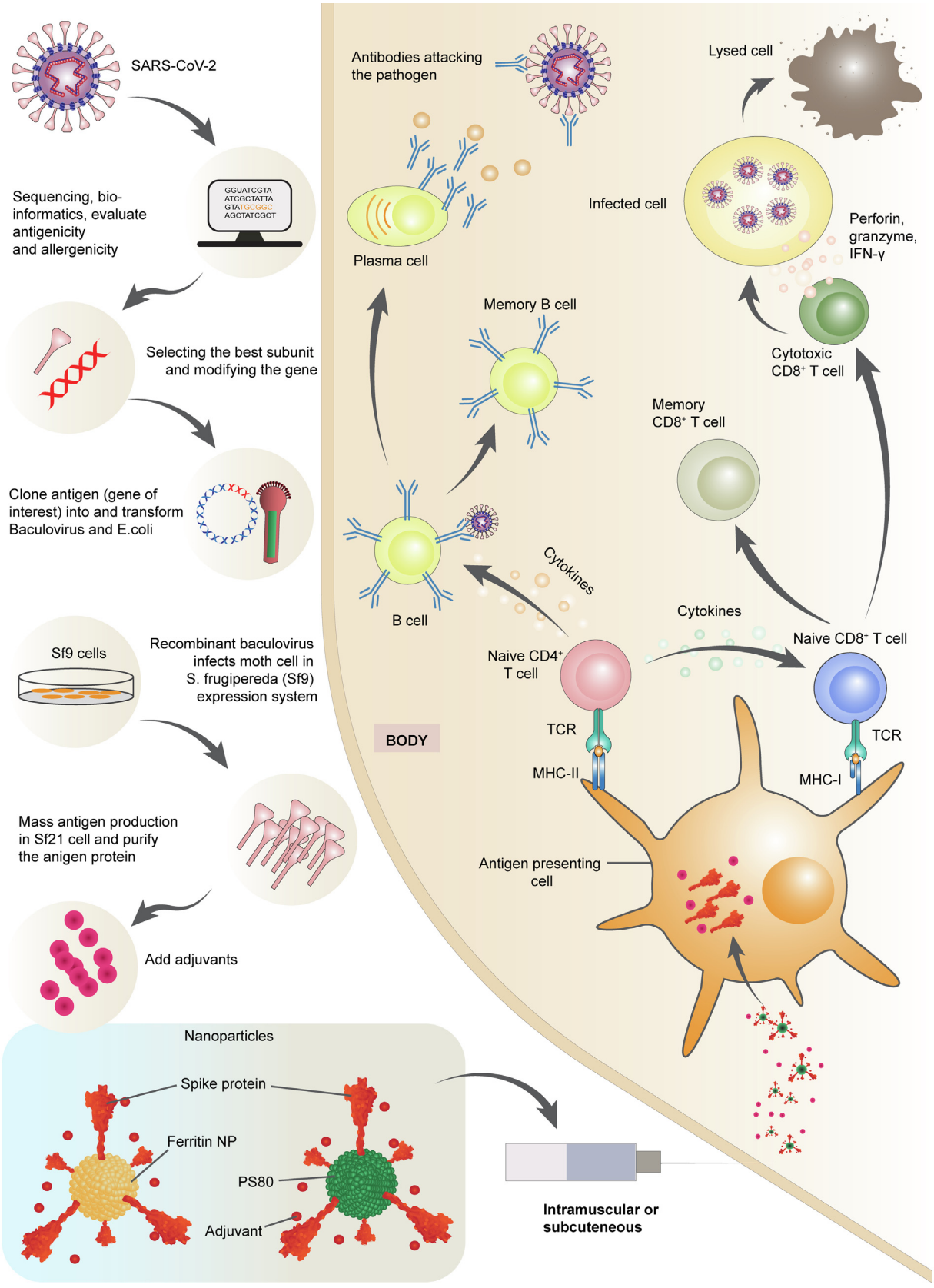


FIGURE 7

Subunit vaccine development and elicitation of the immune response. Identifying, selecting the antigenic protein, and modifying the gene using bioinformatics and computational tools. Cloning the gene of interest and large production of proteins in the expression system. Addition of adjuvants. Intramuscular or subcutaneous administration and immune response generation.

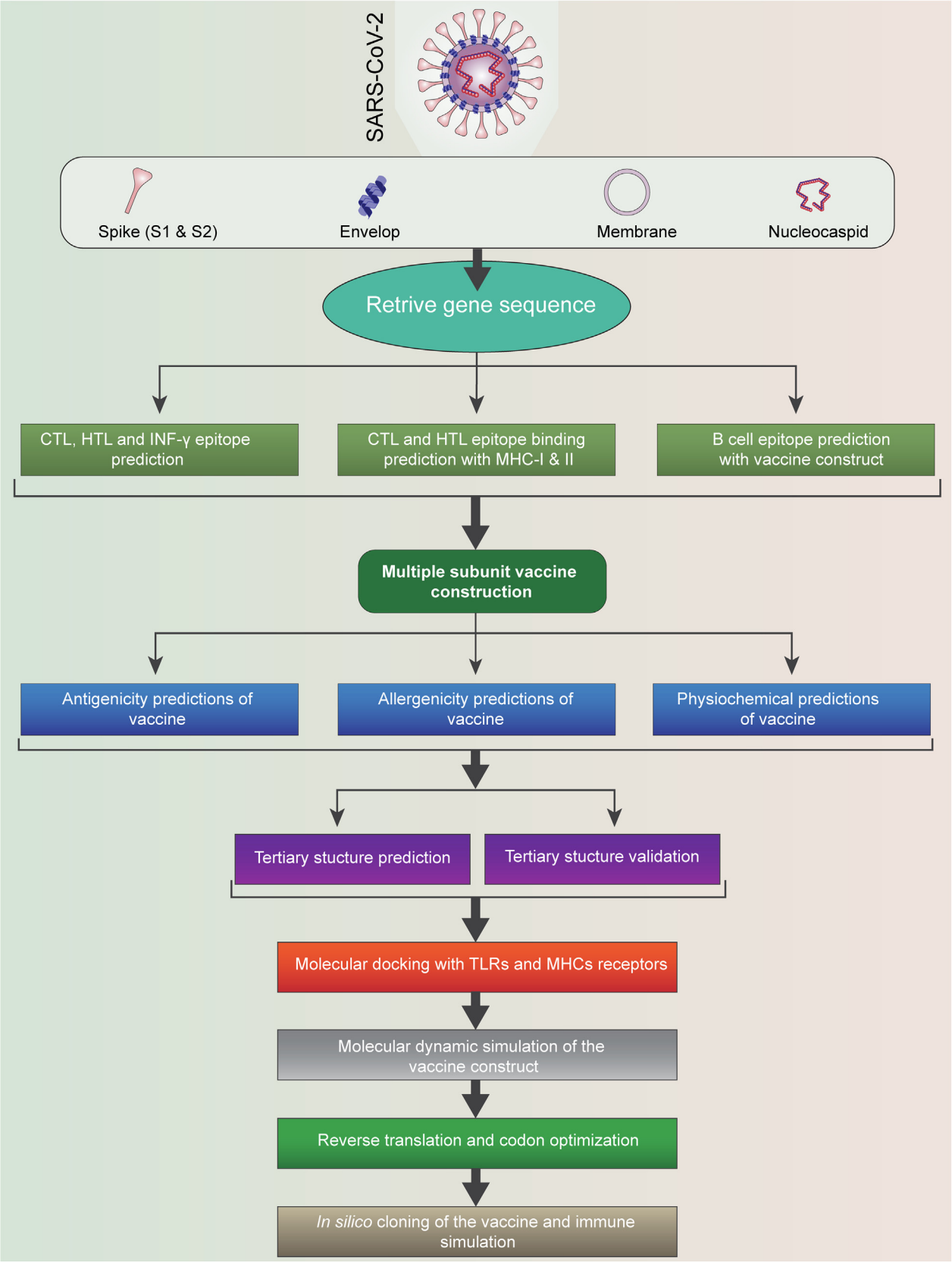


FIGURE 8

Schematic of in silico identification of a suitable epitope/multiepitope-based subunit for vaccine development for SARS-CoV-2.

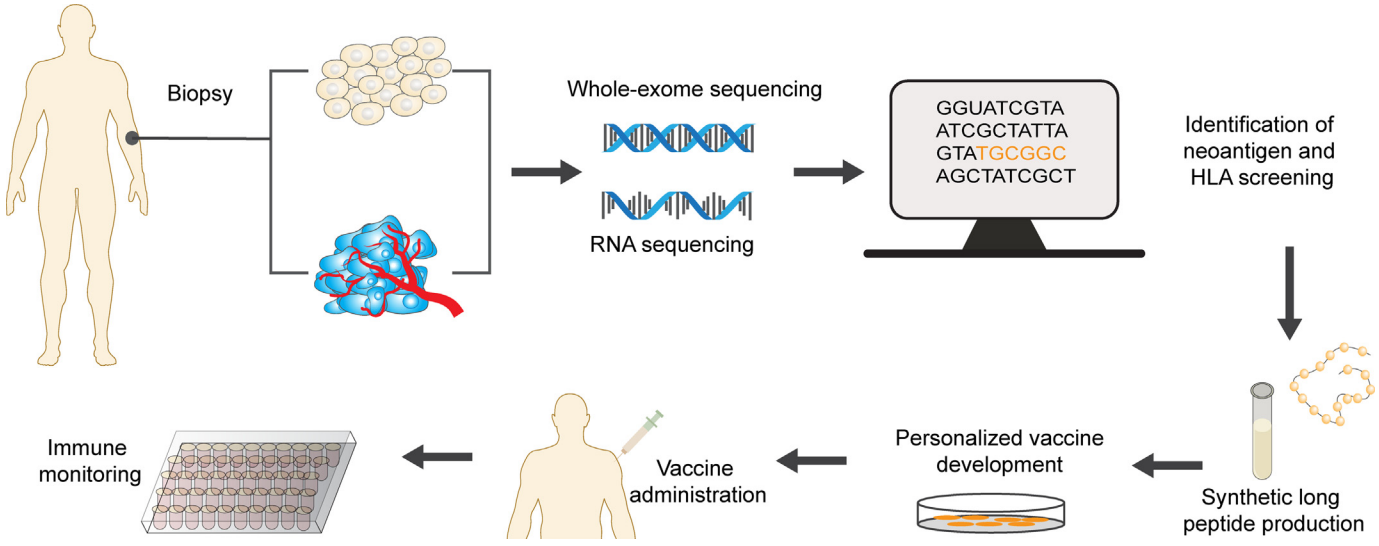


FIGURE 9

Identification of neoantigen for tumor-specific immunotherapy.

cine design and formulation. Immunoinformatic, molecular modelling, and several computational tools made it possible to construct the multiple epitopes and examine the binding to the host protein [182]. Fig. 8 represents the identification of suitable epitope for in silico study. MESVs have already been introduced to several viruses such as Hepatitis C virus [183], Acute Encephalitis [184], Respiratory Syncytial virus [185], Middle East Respiratory Syndrome coronavirus (MERS-CoV) [186] and SARS-CoV-2 [187-190]. The traditional recombinant technology vaccine is formulated on the large protein whole viral genome sequence whereas the MESVs are formulated on short immunogenic responses that also can elicit substantial humoral and cell-mediated immunity against the pathogen. The MESVs has numerous unique features and advantages compared to conventional and typical single-subunit vaccines such as the lower antigenic load and reduced allergic reaction. Another advantage is that T cell receptors of subunit T-cells can recognize multiple major histocompatibility (MHC) Class I and Class II. Moreover, linking adjuvant can induce long-lasting cell-mediated and humoral immunity overlapping the CTLs, Helper T-lymphocyte, and B cell epitope. Furthermore, the difficulty of culturing pathogens and in vitro expression complications can be avoided through this multi-epitope subunit platform [182,191].

The immunoinformatic discipline is crucial in identifying epitopes for the development of a SARS-CoV-2 vaccine. Several in silico studies reported different B-cell and T-cell epitopes, RBD, and spike proteins that are promising in inducing non-toxicity and nonallergic responses with high antigenicity feature combined with adjuvants. However, experiments in the laboratory are needed to confirm their safety and immunogenicity [192,193].

Peptide-based vaccine

Peptide-based vaccines have illustrated enormous potential to combat not only infectious but also chronic diseases. Peptide-based vaccines have lower immunogenicity than conventional vaccines but that can be overcome with the help of some novel technologies like delivering the peptides by lipid nanoparticles and peptide presenting nanoparticles; however, these areas need more research for better understanding [194]. Besides, peptide-based immunotherapy that has the capability to induce both CD4 and CD8 immune responses is possible with the help of bioinformatics to identify specific neoantigens to target the cancer immunotherapy [195].

Neoantigen peptide (neoepitope) is an attractive target for tumor-specific immunotherapy **(**Fig. 9**)**. Neoepitopes are unique entities that are mutated specifically to the tumor that represents "non-self" to the Class I and/or Class II of major histocompatibility [195]. These neoantigens, when combined with nanoparticles, can promote tumour regression and trigger a stronger anti-tumor T cell response. Tumor-specific antigenic T effector cell activation and a potent vaccine platform are required to prevent tumour progression. In an experiment by Arbelaez et al. [196], to improve the delivery of peptides they designed a synthetic long peptide with cationic lipoplexes. This design delivered the peptide to the myeloid cell in the spleen and lymph nodes. They observed the regression of the tumor after elicitation of both $CD4^+$ and $CD8^+$ responses.

Virus-like particles

Virus-like particles (VLPs) are non-infectious, genome-free nanoscale structures that are led by the self-assembled structural proteins of the pathogen. Depending upon the self-assembled structural proteins it can be icosahedral, rod-shaped, or helical in structure [197,198]. Due to the cavity inside their structure, VLPs can be employed in immunotherapeutic, imaging, and as a carrier for the transport of active ingredients and nanomaterials such as drugs, nucleic acid, quantum dots, and imaging chemicals [199,200]. VLPs can be categorized into two forms, Enveloped VLPs and non-enveloped VLPs. These two forms of VLPs can further be classified into single to multi-layered capsid and also single, double, and triple-layered VLPs [201].

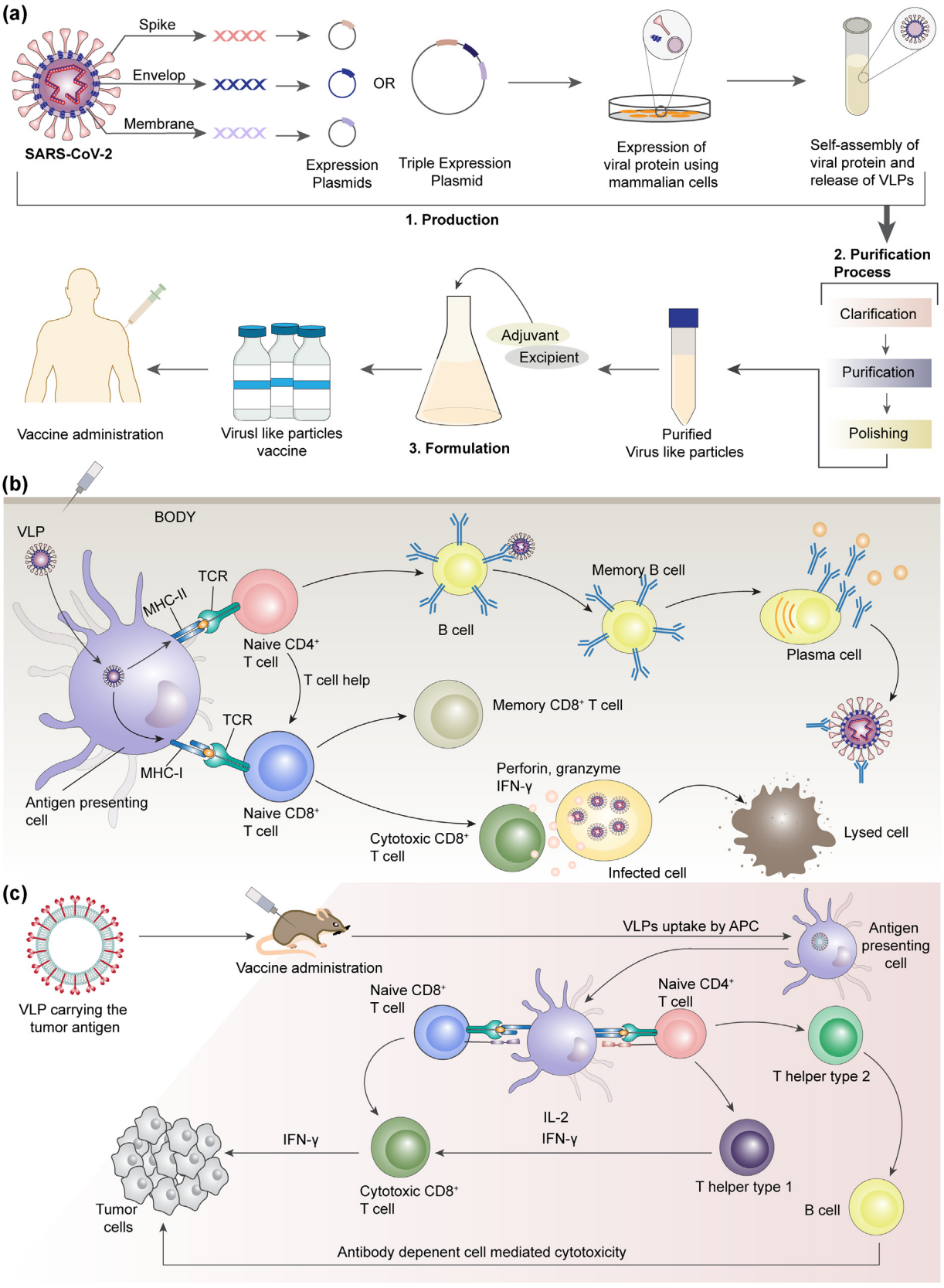


FIGURE 10

Virus-like particles production, and elicitation of humoral and cell-mediated immune response. (a) Expression plasmids using the pathogen's antigenic epitopes (eg. SARS-CoV-2 spike, envelope, or membrane protein) are developed. The viral proteins are expressed in mammalian cells leading to their self-assembly and release of VLPs. VLPs released are purified and adjuvants are added to them for further administration. **(b)** the VLPs administrated into the body induces both humoral and cell-mediated immune response. **(c)** The VLP contains tumor antigens, which upon administration is inducing T cell-mediated and a B cell-mediated cytotoxicity in the body that targets the tumor cells.

Nanoparticles, as an adjuvant and delivery system, offer significant advancements and benefits in the field of next-generation vaccines, such as virus-like particles outperform conventional vaccines in terms of enhanced immune responses against pathogens (Fig. 10 **a and b** represent the vaccine formulation and immune induction mechanism for VLP respectively) [202]. VLPs provide more safety to the immunocompromised elderly people as they resemble in size and shape the native viruses making them more efficient in eliciting immune responses while being genetic material free and non-replicating [203]. Additionally, VLPs can elicit both humoral and cell-mediated immunity in the host body [204-206].

The sudden surge in COVID-19 cases with increased transmissibility and invasion of immune response is a matter of concern. The common mutation in the spike receptor binding domain (RBD) of the virus led to the production of several variants of concern (VOCs) for instance beta, gamma, delta, and alpha. The scope of flexible variation in the size and morphology of the VLPs upon incorporating spike protein and different antigen presentation capability has made it a potential vaccine candidate against SARS-CoV-2 and other coronaviruses [207,208]. In case of SARS-CoV-2, enveloped (eVLPs) and non-enveloped VLPs (neVLPs) have been developed. The role of structural proteins, for instance, membrane (M), envelope (E), spike (S), or nucleocapsid (N) proteins, in the maturation of virus and particle assembly is crucial in the development of the coronavirus-enveloped VLPs. Most particles, however, include the N protein and the highly immunogenic S protein for improved assembly and expression [209,210].

More than seven vaccine candidates for COVID-19 are in the clinical phase of development and 19 candidates are in the preclinical phase of development. Among the seven vaccine candidates, three are in the phase-III from which, an enveloped Coronavirus-Like Particle COVID-19 (CoVLP) candidate developed by Medicago Inc./GSK is leading the race (NCT05040789). The preprint result of phase-II/III revealed that it induced a strong cell-mediated immune response, INF-alpha, and IL-4 in both adult and older adults. Coronavirus-like particle (CoVLP) is a self-assembling VLP with trimers of SARS-CoV-2 recombinant S protein incorporated in the lipid bilayer. These VLPs are made in a plant (*Nicotiana benthamiana*) and have a structure that is very identical to the SARS-CoV-2 virus's native structure [211,212]. The CoVLP vaccine was developed combined with an adjuvant system 03 (AS03) which displayed the S protein of the original SARS-CoV-2 strain. The phase-3 clinical study conducted by Hager *et al*. reported that the CoVLP vaccine candidate showed 69.5% against symptomatic and 78.8% against moderate-to-severe infection of distinct variants (NCT04636697) [213]. The neVLPs lack lipid membranes and can be easily expressed using bacterial and yeast cell expression systems. A neVLP-based vaccine candidate ABNCoV2 capsid VLP (cVLP) developed by Radboud University and AdaptVac is in phase-3 of the clinical trial (NCT05329220). The cVLP, using the split-protein Tag–Catcher conjugation system, acts as an antigen displaying scaffold. The protein antigens for instance, the RBD of the SARS-CoV-2 spike glycoprotein get covalently attached to the cVLP. Furthermore, the antigen-presenting cells, lymph node trafficking, and B-cell activation can all be aided by increased avidity and particle size of the VLP. A licensed oil-in-water emulsion-based adjuvant MF59, with a previous record of safety and efficacy against influenza [214], was conjugated with the cVLP which had a positive neutralizing effect on the SARS-CoV-2 virus [215]. The phase-3 clinical trial report has not been published yet, however, the phase-1/2 trial result showed its effectiveness in mice model. The vaccine was shown to induce higher immunogenicity and neutralizing antibody titer in mice (NCT04839146) [216]. Another VLP-based vaccine candidate LYB001 using aluminium hydroxide as an adjuvant was developed by Yantai Patronus Biotech Co., Ltd. and is in phase-3 of the clinical trial (NCT05664932). The vaccine has a three-dose regimen unlike other VLPs and is expected to be effective in a broad spectrum. The vaccine lacks any genetic material and is a promising candidate against the new crown mutation in variants like delta [217,218]. The phase-3 trial report is not yet published.

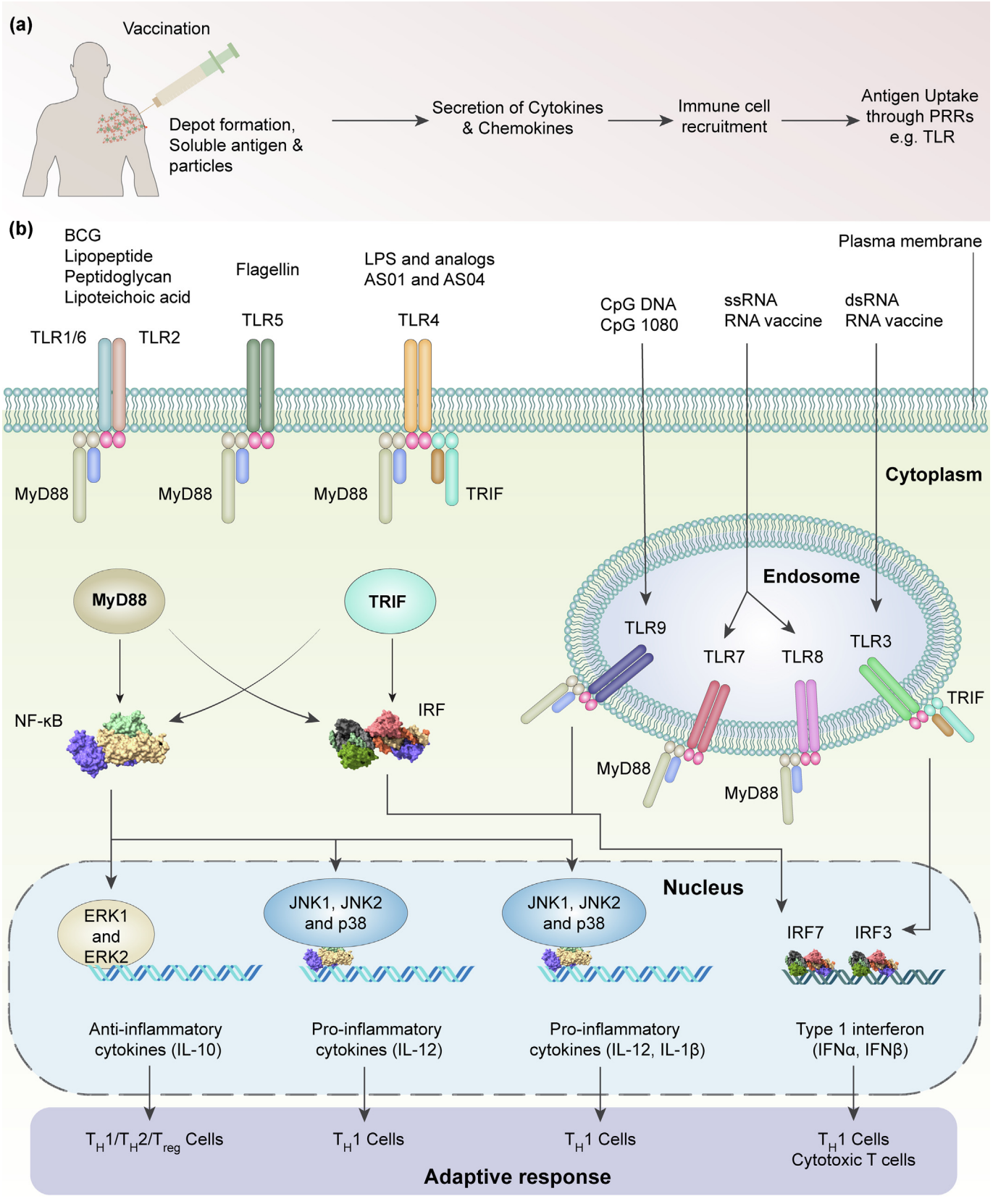
The recombinant viral protein used in developing VLPs is expressed in appropriate expression systems such as plants [219], prokaryotic cells for example *Escherichia coli* [220], yeast [221], mammalian cell line [222], and insect cell line [223]. Moreover, structural protein assembly from different viruses can also construct chimeric VLPs [224]. It is crucial to select a proper expression host for better post-translational modification and protein folding as only mammalian cell provides a better environment [225]. Hence, the production and scalability of VLP remain a challenge to resolve.

VLPs have strategized for four decades as a preventive approach for several diseases including the pathogenesis of human cancer. Regulatory agencies have approved two VLP models, HBV and HPV VLP, for human use only, the rest of the VLP models are in the preclinical and evaluation stage. VLPs are associated with preventing strategies for different cancer-causing pathogens such as Hepatitis B & C virus, Human papilloma virus (HPV), Herpesvirus Type 8, Epstein-Barr virus, etc. Moreover, in association with melanoma, pancreatic cancer, cervical cancer, and hepatocellular carcinoma therapy, VLPs have demonstrated an efficient platform in presenting tumor-associated antigens to the specific immune cells (Fig. 10 **c**). With all the advantages, several issues remain associated with production, scalability, and immune generation. Dominant targeting of one arm of the immune system could be the reason for an inability to induce sterile immunity and total clearance of the oncogenic virus [226].

Calcium phosphate nanoparticle

In vaccines, formulation adjuvants are the essential component to induce robust immunity again infectious agents. In viral immunity and cancer immunotherapy, the challenging goal is to induce a robust and specific CTL response. In cancer therapy, the microenvironment adds up more complications in CTL response due to the immunosuppression of T cells via several pathways. Increasing the binding of the vaccine to the host cell and delivering the antigen cargo through the endocytic pathway of the antigen-presenting cell is the key factor to enhance the efficacy of a vaccine. Toll-like receptors, expressed on the APC and mucosal/oral epithelial cells, are a group of transmembrane protein complexes that recognizes the microbial components. These protein complexes are called pathogen-associated molecu-

lar patterns (PAMPs) (See Fig. 11a). Because of their ability to increase the efficacy and immunogenicity of vaccine formulations, TLR agonists as adjuvants have gained increasing attention [227-229]. Immune cells recognize the pathogens through the TLR which leads to increased antigen uptake, pro-inflammatory cytokine, and chemokines and co-stimulatory molecules (CD80, CD86, CD40) expression which results in a robust elicitation of innate and adaptive immunity. Co-immunizing an anti-



gen with a TLR ligand has the advantage of integrating mechanisms that up-regulate the expression of co-stimulatory molecules and induce pro-inflammatory cytokines, which can be used to overcome the immunosuppression of Immune cells in cancer therapy [230,231] (See Fig. 11b).

Aluminum salt is the most widely used adjuvant that can elicit Th2-biased humoral immunity in humans with high antibody titer but with a limited response. Calcium phosphate nanoparticles (CaP-NPs) are new generation vaccine adjuvant that has the potential to induce both Th1 and Th2 balanced immune response. CaP-NPs have a tuneable property and are biocompatible however, the biocompatibility of CaP-NPs necessities further research to develop a human vaccine [232]. Calcium, phosphorus, and oxygen, three chemical elements that are abundant on Earth's surface and in biological systems, constitute CaP-NPs. Calcium phosphate, a naturally occurring component of the body, is easily reabsorbed in living cells and is well tolerated [233,234]. It has been demonstrated that CaP adjuvants protect the antigen payload from premature enzymatic and proteolytic degradation and hinder the reticulohistocytic system from eliminating it [235]. Additionally, various forms of TLR molecular structure regulate the properties of CaP NPs and their immunostimulatory function. The mineralization of CaP NPs is controlled by the backbone, sequencing, concentrations of CpGs, and composition [236]. A study targeting the CaP-NP for oral vaccine delivery was conducted by Cao et al. The CaP-NP was coated with chitosan and alginate. In oral administration, the major challenge is to protect the antigen from acidic degradation in the gastrointestinal environment. The mucus barriers and the insufficient uptake of antigens by the immune cell are also associated with the key challenges in oral administration. The CaP-NP was prepared via a water-in-oil-microemulsion method and subsequently coated with chitosan an alginate. The formulation demonstrated promising results in delivering the antigen. The 50 nm size CaP-NP alginate coating protected the antigen from degradation while sustain release was observed at pH 6.8 and 7.4 of the antigens due to the chitosan coating. The chitosan coating also enhanced the antigen uptake in the Caco-2 and macrophages. The costimulatory molecules were observed to be highly expressed on the macrophage surface due to the chitosan coating. Moreover, the serum IgG antibody and mucosal IgA antibody response were significantly enhanced after the oral administration of the formulation in the mice [237].

Nanoparticle interaction with immune cells and antibody activation

Understanding B cell and T cell interaction with soluble or particulate vaccine antigens is the fundamental step in understanding the induction of antigen-specific long-term immunity. Few parameters such as shape, size, and composition of nanoparticles greatly influence the uptake of nanoparticles through binding of surface or endocytosis pathway [238].

The persistent antibody could be seen in SARS-CoV and MERS-CoV for 2–3 years in critical infected individuals however in COVID-19 lack of lymphoid structure is reported in the post-mortem reports. The lymphoid organs are responsible for producing a durable antibody that fights against the pathogen. In the lymph node within the follicular B cell, the antigen primed B cell colony expands after encountering the antigen. The naïve B cell directly encounters the antigen by immature BCR in the follicular B cell or on the surface of resident APC. The immunoglobin gene of B cells undergoes secondary diversification after clonal expansion selecting and producing plasma and memory B cells [239].

Somatic hypermutation and V(D)J recombination are the major processes in humans responsible for a diverse antibody repertoire and a rapid humoral response against a variety of pathogens. Furthermore, primary diversification is accountable for a significant amount of the diversity in the antibody repertoire. However, sometimes it's difficult to produce an enhanced diverse antibody repertoire against several bacterial and viral pathogens, which requires secondary diversification. The secondary mechanism is critical in D-D fusion in V(D)J recombination making V(DD)J recombinant, somatic hypermutation associated insertion and deletion, antigen contact by non-CDRs (complementarity determining regions), and affinity maturation that contribute to enhanced antibody repertoire diversity [240].

The nanovaccine localization and carrying traffic of antigen to the follicular B cell to encounter follicular Dendritic cells is a critical step in inducing potent immunity. The nanoscale size range of the nanocarriers is an important vehicle design parameter that can determine the spatial localization of antigen. The nanovaccines enter the lymph nodes through the afferent lymphatics and after being picked up by lymphatic fluid, leave the lymph nodes near the medullary sinus through efferent lymphatics. The antigens are localized at different regions inside the lymph nodes, particulate antigen localizes at the subcapsular region macrophages covering the B cell follicles. Nanovaccines with antigen, less than 200 nm, enter directly through the afferent lymphatics and resides outside the B cell follicles **(**Fig. 12 **a)**. Nanovaccine also targets the inner structure and dendritic follicles for instance the glycoengineered nanovaccines. The composition, different sizes, and shape of nanovaccines made it possible to target the inner structural component however it is not a common characteristic of nanovaccine. The small hydrodynamic radius nanovaccine of about 5 nm finds a direct entry to

FIGURE 11

Mechanism of action of adjuvant. (a) In some cases, depot forms after vaccination due to the release of particles and soluble antigen. Immunocompetent environment forms at the injection site due to the secretion of cytokines and chemokines which recruits various immune cells. Then pattern recognition receptors such as Toll-like receptors uptakes the antigen. (b) Adjuvants that activate TLRs and the induced signalling pathway. The targets of the depicted adjuvants include cell surface TLRs (TLR1,2,4,5,6) and endosomal TLRs (TLR3,7,8,9). TLRs recruit adaptor proteins with TIR domains, such as MyD88 and TRIF, upon the proper adjuvant being recognized by the leucine-rich repeat domain. These proteins then activate NFκ-B signalling, MAP kinases, and IRFs (e.g., IRF3, IRF7 mediated type I IFN responses). Pro-inflammatory cytokines such TNF-, IL-6, IFN-, or Type I interferons IFN- and IFN- start expressing themselves as a response.

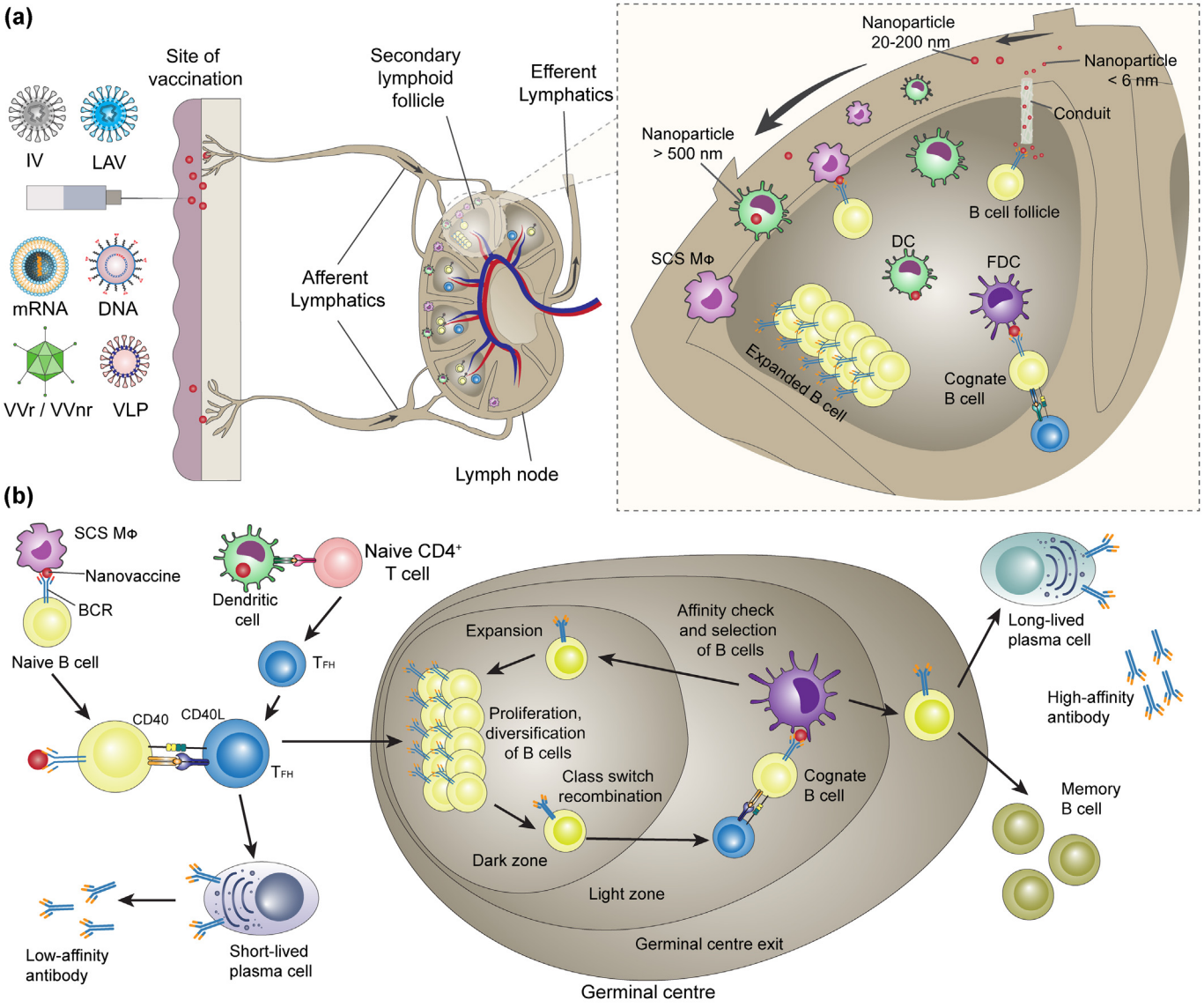


FIGURE 12

Administration of conventional and nanovaccine at the vaccine site and their interaction with different immune cells. The vaccine particle reaches the lymph node through afferent lymphatics and in LNs it interacts with different presenting and immune cells. Nanovaccine sometimes forms a depot at the vaccine site of injection for slow release (a). The naïve B cells interact with the subcapsular macrophages. The interaction of T_{FH} and CD4 cells stimulates the proliferation of B cells germinal center's dark zone. After, affinity check ad selection, B cells developed into long live plasma cells and memory B cells (b).

lymph nodes through a collagen fiber-rich network conduit which is prevalent between the B cell follicles to the T cell zone [239,241,242].

Though the conduit has a 1 μm wide opening it allows the passage of approximately 70 kDa and <5 nm dynamic radius sized nanovaccine due to the small 10 nm passage of collagen fiber. The larger size nanoparticles of 200–500 nm get proteolyzed and drain into the subcapsular sinus through macrophages or by the conduit. Dendritic cell internalizes the larger size nanoparticle of >500 nm through FcγRIIb, Fc fragment of IgG receptor, that is later presented to B cell [238,243]. Chemokines are crucial in the migration of APCs via single lymphatic endothelial cells. Lymphatic endothelial cells are the main barrier for antigen-containing APCs mainly DCs during the migration through the subcapsular sinus to the T cell resident cortex zone. Large APC cell size restricts the migration and passage, however certain chemokines, CCLR1 expressed on lymph node periphery generates driving force of CCL19 and CCL21 concentration gradient formation which initiates the migration of DCs toward the cortex region resident T cell for specificity [242].

The germinal center (an anatomical compartment at B cell follicles) response, is one of the critical factors in inducing durable and higher affinity antibodies against any antigen. The primed B cells interact with the Tfh at the border of the B cell follicles where the CD40L of Tfh and CD40 of B cell binding occurs. Depending on the binding B cell differentiate into short-lived plasma cells or specialized germinal centers **(**Fig. 12 **b)**.

After immunization, within 7–10 days B cell proliferates through a controlled epigenetic and transcriptional process as the germinal center grows and diverges into dark and light

regions/zones. In the dark zone of the germinal center B cell proliferates rapidly and undergoes somatic hypermutation to produce higher affinity diversified antibody repertoire clones [239].

B cell clones then migrate to the light zone where follicular dendritic cells are already localized presenting the foreign antigen to test B cell receptors (BCR) affinity toward the antigen. In the selection process, some B cells get enough activation or undergo apoptosis, whether a few migrate back to the dark zone for further mutation and proliferation. Somatic hypermutation at the variable region where antigen binds to selected B cell immunoglobin gene leads to the production of high-affinity Immunoglobin-α (IgA) and Immunoglobin-γ (IgG). B cells that have been activated exit the light zone and leave the germinal centre shortly after, eventually differentiate into long-lived antibody-producing plasma cells and memory B cells. Antibodies released from long-lived Plasma B cells relocate to the bone marrow, and protect from reinfection for months, years, and sometimes for a lifetime. The memory B cell does not secrete antibodies but differentiates into plasma blast as in case of reinfection [239,241].

Antigen accumulation in the lymph node depends on the antigen dose and plays a critical role in potent immunity induction i.e., B cell and T cell activation depends on the antigen dose [244,245]. Moreover, the level of antigen influences T cell differentiation.

The expansion of cytotoxic T lymphocytes and T helper cells is determined and limited by the presence of APC in the lymphocyte bearing the antigen. Preclinical studies suggested that CD8+ activation requires a large amount of antigen. The disturbance or low elicitation of CD8+ T cells can cause further disturbance in the antigen processing and cross-presentation [241,246]. A higher level of accumulation of antigen in the lymph node produces a greater number of follicular helper T cell that eventually regulates the germinal center [247].

In the case of cancer immunotherapy, vaccines take around 3–7 days to reach LN for the highest accumulation of antigen to encounter APCs and quickly active lymphocytes however most of the antigen-bearing APCs cannot reach the lymphoid organs. This period of activation of immune cells is sufficient for tumor cells to increase their volume by 8–10 times as seen in mice. Hence, slow immune induction is the major limiting factor in limiting cancer immunotherapy efficiency. Administration of nanovaccines, through subcutaneous or intramuscular route, makes a vaccine depot at the injection site where retains for months to gradually stimulate a durable immune response. Due to the alum and emulsion depot, some side effects happen such as local inflammation, hemolytic activity, and apoptosis of T cells. To overcome the limitations of conventional vaccines or adjuvants (alum and emulsion), nanoadjuvants (hydroxide and hectorite) have been widely investigated due to their controllable physicochemical properties such as size, shapes, hydrophobicity, and light interaction and have been seen to induce a potent immune response in cancer immunotherapy [243].

Nanoparticle based vaccines to overcome challenges of available and pipeline vaccines

Influenza

Influenza is a severe contagious epidemic disease that infects the respiratory tract causing approximately 3–5 million severe cases and 290,000–650,000 fatalities around the world. Influenza can escape established immunity due to its high seasonal antigenic drifts, mutation, and viral ability which is a significant problem in public health. Currently licensed influenza vaccine (inactivated, live attenuated, and recombinant HA) requires an almost annual update in the formulation to induce specific NAbs targeted to the hypervariable epitope. Moreover, systemic, and local side effects, adverse effects due to impurities, low immunogenicity, and reactogenicity are some limitations that make the influenza vaccine less efficient which indicates the necessity of a potent or universal vaccine. The hemagglutinin, neuraminidase, M2, M1, and nucleoprotein epitopes are conserved proteins that can be targeted to formulate a universal vaccine. Moreover, across the different influenza variants, the stem or stalk region of hemagglutinin is highly conserved and considered a promising target [248-250].

Several nanomaterial-based vaccines are under development for a promising protective influenza vaccine. Different nanoparticles such as lipid nanoparticles [251], gold [252,253], ferritin [249,254], graphene oxide [255], self-assembly nanorods [256], recombinant proteins [257], are used for the construction of universal influenza vaccine incorporating HA, M2e, nucleoproteins. All these nanoparticle-based influenza vaccines have shown promising results in protecting different animal models, including mice, ferrets, and macaques.

Gold nanoparticles incorporating M2e antigen-based vaccine has demonstrated to be thermally stable at 4 °C for 3 months, 37 °C for 3 months, and 50 °C for 2 weeks in a mice model. Further, Rohan *et al.,* stated that the thermally stable nanovaccine can greatly reduce the economic burden. It has been established that as AuNP size increases, so does the wavelength at which a colloidal solution of AuNPs exhibits its maximum absorbance. After being resuspended in water, they noticed that the UV–vis spectra for all formulations stored at 4 °C, 37 °C, or 50 °C were consistent, even though their peak wavelengths were higher than those of a freshly prepared formulation. Additionally, the hurdle of degradation of the main immunogenic component of Influenza from high temperatures can be resolved to a great extent. The intranasal vaccine was formulated and stored in dry form and can be resuspended in water with the ease and convenience of self-administration through the intranasal route that will help in mass vaccination [253].

In a unique approach, mosaic nanoparticles using the RBD of HA are used that elicited broader antibody response, B cell cross-reactivity, and specific monoclonal antibody preferentially a novel immunological pattern in mouse [258]. This immunosubversive approach is achievable through advanced protein engineering and manufacturing in combating pathogen that evades immunity by exploiting genetic plasticity and genetic variations. This modular self-assembling nanoparticle platform designed on the ferritin nanoparticle scaffold was created to construct the mosaic nanoparticle. With the help of this technique, antigen heterogeneity and homogeneity that was shown as an array on the assembled nanoparticle could be manipulated. From a fusion construct connected to an engineered ferritin sequence, monomeric RBDs were produced. Transfected cells that have RBD-np expressed in them spontaneously self-assemble to construct particles that are released into the culture supernatant. This tech-

nique allows the production of both heterogeneously co-assembled mosaic RBD nanoparticle and homogeneously assembled RBD nanoparticles.

A saponin-based Matrix-M adjuvanted recombinant hemagglutinin nanoparticle influenza vaccine Nanoflu™ is in phase 3 of a clinical trial (NCT04120194). In phase 1 clinical trial it demonstrated board cross-reactive protective antibody response results and two broadly neutralizing monoclonal antibodies A2.91.3 and A2.4.1 were derived from vaccinated mice against A(H3N2) influenza strains [257]. The recombinant hemagglutinin nanoparticle vaccine was developed on a PS80 detergent/protein micelle core structure where the purified HA trimers hydrophobic transmembrane region interacts with the PS80 (polysorbate 80 or tween 80) that constructs the nanoparticle [259]. Protein is a highly surface-active compound and quickly adsorbed due to the hydrophilic, hydrophobic amino acids and chemical side chain moieties the leads to the denaturation and aggregation of the protein at the interface. On the other hand, polysorbate being an amphiphilic molecule competes with the protein for interface and prevents the interface-induced aggregation on the protein. Hence, polysorbate is integral to antibody and other protein-based formulations [260].

Ferritins are protein-based nanocages with the property of self-assembling that makes this nanoparticle more interesting in vaccine development. Ferritin nanoparticles are naturally derived biocompatible which mimics both size and structure of the pathogen and are amenable to surface conjugation that promotes the interaction with immune cells [261]. A self-assembling ferritin nanoparticle targeting stem HA surface glycoprotein recognized to elicit protective and homosubtypic antibody and activating unmutated ancestral B cell receptor of broadly neutralizing antibody is in phase 1 clinical trial (NCT03814720) [254]. A protein engineering strategy was used to stabilize group 1 and group 2 HA stem immunogen. Without helix stabilization, loop optimization and side chain repackaging the self-assembling ferritin nanoparticle was not stable.

A lipid nanoparticle-based hemagglutinin stalk mRNA vaccine (universal) has been reported to induce stalk specific antibody response in mice, ferrets, and rabbits protecting from homologous, heterologous, and heterosubtypic influenza virus infection in mice that has the potential to replace current vaccines in terms of rapid production and safety profile. The phage RNA polymerase mediated in vitro transcription of DNA is the most efficient technique however, it produces large quantities of RNA, and the unwanted activities of polymerases contains impurities. The HPLC method is beneficial in scaling and removing multiple contaminations from the in vitro transcribed RNA. The nucleoside-modified and fast protein liquid chromatography purified mRNA lipid nanoparticle was demonstrated to elicit potent antibody response targeting the conserved HA stalk domain of influenza virus in all three animal models [9].

One of the lipid-nanoparticles-based HA mRNA vaccines has completed its phase 1 clinical trial. The result demonstrated that the 2-dose mRNA vaccine elicits a robust humoral immune response whether the cell-mediated immune response was not significant [262]. This formulation was independent of the exogenous RNA to act as an adjuvant or on adjuvant properties during the self-amplification of the mRNA. While using the lipid nanoparticle the formulation could produce a high level of transient expression without the necessity of immunostimulatory compounds [263]. In another study, mice were injected with an mRNA vaccine targeting the H3N2 influenza virus's HA gene, which elicited both humoral and cell-mediated immunity. The lipid nanoparticle for gene delivery was modified with a mannose ligand that improve the efficacy of the delivery. According to the ratio of nitrogen on DOTAP and phosphate on mRNA lipid nanoparticles/mRNA were prepared [264]. A similar study targeting the multiple conserved antigens (hemagglutinin stalk, neuraminidase, matrix-2 ion channel, and nucleoprotein) of the current seasonal H1N1pdm variant by modifying nucleoside mRNA carried by lipid nanoparticles has been carried out. The result illustrates the strong induction of humoral and specific elicitation of CD4+ and CD8+ immune response in a murine model. A single-dose immunization could protect against a lethal dose of Influenza A and H1N1 strain [265].

The mRNAs or lipids can be integrated with hydrophilic and hydrophobic adjuvant molecules to produce mRNA LNPs with core-incorporated or surface-anchored adjuvants. In recent years, several vaccine investigations have used 2′3 cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) alone or in conjunction with other molecular adjuvants. It has been demonstrated that cGAMP, a naturally occurring STING agonist with a negative charge, functions effectively as an adjuvant for influenza vaccines [266,267]. The immunogenicity of mRNA LNPs was significantly enhanced by the addition of cGAMP. Higher CD4+ resident memory (TRM) T cells in the lungs and increased IL-4 and IFN- secreting cells and effector memory T cell populations in the spleens have been seen in the cGAMP mRNA LNPs immunized group of mice [268].

Human Immunodeficiency Virus/ AIDS

HIV is one of the most prevalent viruses around the globe that lead to acquired immunodeficiency syndrome (AIDS) if left untreated. Since the start of the HIV epidemic average of 79.3 million people have become infected and 36.3 million have died. In the year 2020 globally 37.7 million people were living with HIV, 53% were women and girls, and 680,000 individuals died due to HIV infection [269].

Early studies have demonstrated that HIV-1 specific CD8+ cytotoxic T lymphocyte plays a protective role in viral control in the acute phase of infection and potent polyfunctional cellular immune response [270,271]. However, the sterilization immunity to prevent infection was not achievable with robust elicitation of cellular response. Several immunological and virological factors such as error-prone reverse transcription-based highly dynamic genome of HIV-1 and recombination between genome copy stands in the way to develop an efficient vaccine [272].

The elicitation of bNAbs is critical and a priority in HIV-1 vaccine formulation due to its high level of somatic hypermutation, poly-reactivity, and long complementary determining regions 3. bNAbs are induced by subsets of untreated individuals during the period of infection but currently it's difficult to induce by passive immunization through a vaccine. The most variable and minimal immunogenic glycan fence are extensively present in the Env that acts as a decoy to direct away from the humoral

response from the bNAbs target relative constant regions. Hence, it is extremely difficult to design vaccine immunogens capable of recapitulating the bNAbs process [272]. Earlier clinical trials of STEP and RV144 vaccine results were disappointing but provided a paradigm shift in the vaccine design. The STEP trial strategizes to examine the cell-mediated efficacy but failed to prevent the infection or viral load. Though, RV144 was effective to 31% of participants, illustrated the immunological correlation of $CD4^+$ T cells and Fc mediated antibody response in protection from HIV acquisition, and provides a basis to design a next-generation vaccine regime [273,274].

A promising approach with mRNA vaccine co-expressing HIV-1 envelop and Gag protein of simian immunodeficiency virus that generates VLP in rhesus macaques induced broader robust neutralization antibody accompanied polyfunctional CD4+ T cell response. The co-formulated mRNA vaccine was safe and immunogenic to protect rhesus macaques with high neutralization breadth and in mice, env protein was alone superior to inducing NAbs [275]. Immune complexes and self-assembling protein nanoparticles are demonstrated to persist in lymph nodes targeting the follicular dendritic cells in the nascent germinal center's light zone [276]. A self-assembling lipid-nanoparticle mRNA-based HIV vaccine is developed targeting the outer domain of gp120, the main receptor of the HIV-1 virus. The vaccine is currently in phase 1 of a clinical trial (NCT05001373). The study hypothesized that sequential germline-targeting prime immunization followed by directional immunogen boots through an mRNA platform can elicit and guide a specific class of B cells to early maturation capable of evolving towards bNAbs [277,278].

The viral components as adjuvants can be beneficial, compared to bacterial adjuvants in enhancing vaccine efficacy and uptake. A study conducted by Xu *et. al* evaluated the potential of P1, a conserved amino acid peptide that covers the Membrane Proximal External Region (MPER) of HIV-1 envelope subunit gp41, *in vitro*. The intranasal vaccines, targeting nasal epithelial cells and dendritic cells, against mucosal HIV infection with P1 peptide was previously seen to be effective as a vaccine antigen that provided full protection. The P1 adjuvant was seen to be activating mucosal dendritic cells leading to Th2 cytokines and chemokine secretion that can induce mucosal humoral responses [279,280]. Another study conducted by Barnowski *et al.,* on the effectiveness of flagellin protein, in HIV vaccine development as an adjuvant, showed that it induced innate immune response because of its affinity towards toll-like receptor 5 (TLR5). The study investigated a truncated membrane-bound form of the flagellin protein KFΔ adjuvant combined with an HIV-based VLP vaccine. A few other studies have also demonstrated the ability of flagellin adjuvant in recruiting B cells and T cells to secondary lymphoid sites, and activation of T lymphocytes and dendritic cells [281,282].

Malaria

Malaria is an acute illness caused by a protozoan parasite *Plasmodium* spp. That is transmitted through infected female anopheles' mosquitoes. According to WHO the total number of cases of malaria by 2020 was around 241 million and the number of deaths was around 6,27,000 globally. The African regions carry high proportions of global malaria load and in 2020 it was estimated to home around 95% of cases and 96% of deaths of malaria [283]. Over the past two decades, more than 40 malaria vaccines are in pre-clinical and initial Phase-I and Phase-IIa of clinical trials with continuous effort to induce an effective long-term immune response against the *Plasmodium* spp. [284]. But only one vaccine candidate RTS, S/AS01, under the brand name Mosquirix, is approved by WHO in 2021 for malaria prevention. The failure of proper vaccine development against malaria might be caused by the ability of *Plasmodium spp*. to evade our immunity because of its complex life cycle and ability to mutate a wide range of polymorphic proteins that generate new strains. Most of the vaccine candidates in development including Mosquirix target the sporozoite stage of the parasite life cycle which is the entrance phase of the parasite to the human body from the mosquito host [285,286].

A study, tracking the *Plasmodium falciparum (Pf)* specific B memory cells (B_m), reported that over a year the number of B_m cells increased after acute malaria and post six months of *Pf* exposure that decreased to a point slightly higher than pre-infection level. This loss of B_m cells could explain why the vaccines fail to induce robust antibodies in the long term [287]. $MSP1_{19}$, a high titer of protective antibody and vaccine-specific B_m cell producing vaccine candidate that was in clinical trials in Kenya couldn't protect against malaria and was short-lived in the malaria-infected mice model [288,289]. Furthermore, evidence of *Plasmodium* spp. Compromising the dendritic cells (DCs) function that is required to induce long-term immune response has also been found [284,290].

Few recent advancements in vaccine delivery systems using different approaches like using self-amplifying RNA, VLPs displaying circumsporozoite protein (CSP), and nanoparticles are considered nowadays (See Table 3). Using nanoparticles as delivery systems have taken the malaria vaccine development to a next level [291]. The nanoparticles approach considered in the case of malaria vaccine development is using the self-assembling protein nanoparticles (SAPN). This technique involves the manipulation of peptides and proteins that allows them to self-assemble into chemically and mechanically stable particles, unlike the linear unstable peptide antigens that break down easily instead of generating an effective immune response [292]. For instance, the combination of a *P.Vivax* circumsporozoite protein (CSP) repeat peptides and T cell epitopes of PfCSP led to a self-assembling protein nanoparticles **(**SAPN) construct, that induced an effective immune response against the PfCSP bearing sporozoites. PfCSP antigen is used for different vaccine development because it is the immunodominant coat protein of the malaria parasites' invasive stage [292]. Along with stability, the capability of multiple insertions of epitope targets has an advantage over other techniques. In the case of the malaria parasite, antigens from two B cells and different life cycle stages can be targeted to generate immune responses. For instance, a multivalent vaccine candidate developed including domains of *P. falciparum* CSP, CD4+, and CD8+ epitopes, and universal Th cell epitopes among others are tested in clinical trials after it generated a protective immune response in mice model (NCT04296279) [291].

TABLE 3

Nanoparticles in dengue, respiratory syncytial virus, herpes simplex virus vaccine development targeting specific antigens.

Disease	Vaccine platform	Nanoparticle	Antigen	Dose	Animal Model	Reference
Dengue	DNA	Cationic lipid nanoparticle as Adjuvants	Membrane, Envelop protein	3 Phase 1 (NCT00290147)		[306]
	mRNA	Lipid nanoparticle	Membrane and envelop structural protein	3	Mice	[307]
	mRNA	Lipid nanoparticle	prME, E80, or NS1	3	BALB/c Mice	[308]
	Tetravalent Subunit	Cationic lipid nanoparticle	DEN-80E (Envelop protein)	3	Mice, Guinea pigs, and Rhesus macaques	[309]
Respiratory syncytial virus	mRNA	Lipid nano particle	Fusion protein	2	Rodent	[310]
	Self-amplifying	Ferritin	Pre-Fusion protein	2	Mice	[311]
	Epitope focused vaccine	Nanoring	Palivizumab targeted epitope Fusion protein	2	Mice	[312]
Herpes Simplex Virus	mRNA	Lipid nanoparticle	Glycoproteins C, D, and E	3	Mice	[313]
	DNA	Fe_3O_4, coated with glutamic acid	glycoprotein D antigen and interleukin-21	3	Mice	[314]
	Subunit	Calcium phosphate nanoparticle	glycoprotein	2	Mice	[315]

The major symptomatic infection-causing stage of malaria in the blood stage and eradicating the parasite before or at this stage can act as a therapeutic strategy. The vaccine candidates MSP4 and MSP5 target the blood stage of malaria as their antigen diversity is limited for *P.falciparum* and *P.Vivax*. Also, they have shown polymorphism only in specific gene regions in comparison to other high polymorphic vaccine candidates, which makes them a suitable candidate [83]. Vaccines targeting blood-stage malaria generally aim to produce neutralizing antibodies to restrict the parasites from entering the RBCs, along with inducing IFNγ produced by Th1 CD4+ T cells or IL-4 produced by Th2 cells that provide B cell help. The Plasmodium yoelii produced antigen MSP4/5 of murine blood-stage conjugated chemically to pullulan-coated iron oxide nanoparticle (pIONPs) induced antibodies in mice. The biodegradable non-toxic nature of pIONPs and the capability to avoid the production of pro-inflammatory cytokines that differs from traditional adjuvants like alum or CpG can be explored in the future for making an effective malaria vaccine [293].

An mRNA vaccine developed for malaria used the PfCSP antigen for assessing its potential immune response. For a protected delivery of mRNA into the translational machinery of the cell and adjuvant supply stimulating Tfh cells, encapsulation by lipid nanoparticles (LNP) was used. Along with protected delivery of mRNA from extracellular ribonucleases, the LNPs facilitated cell uptake by endocytosis. This study for pre-erythrocytic malaria assessment showed that the PfCSP mRNA vaccine was a compelling candidate as it induced protection in mammalian cells, rodents, and mice models. With several factors inducing protective immunity, the mRNA vaccine is suitable for future investigation [294]. In addition to these, the self-amplifying RNA vaccines don't require freezer storage and are easier to deliver into malaria-endemic areas unlike the mRNA vaccine used for COVID-19 [291].

Tuberculosis

The drug-resistant *Mycobacterium tuberculosis* (Mtb) bacterium has created havoc throughout history due to the lack of efficient vaccines against it. According to the World Health Organization, tuberculosis infected 10 million people in 2017 and killed an estimated 1.3 million, leaving it one of the major causes of mortality from a single infection. Bacille Calmette Guerin (BCG) is the only tuberculosis vaccination used in TB-endemic countries [295].

A meta-analysis reported that the BCG vaccine generates 50% protection against TB for almost 10–20 years. The immune response generated by BCG in adults is much lower in comparison to children. This is a hypothesis suggested by [296] regarding the fact that effector memory T cells (T_{EM}) generated by BCG could protect up to 10–15 years. The efficacy varies with environmental conditions for example, in India, Indonesia, and other warmer countries efficacy is much lower in colder countries like UK and Norway. The major factors influencing lower efficacy rates are sensitization to environmental mycobacteria and prior infection with Mtb. The generation and recruitment of $CD4^+$ and $CD8^+$ T cells that produce type-1 cytokines IFN-ɣ and TNF-α to lungs provide protective immunity to Mtb. Tissue-resident memory T cells (TRM) residing in the lungs produce immunity against Mtb. Two ongoing major approaches to overcome the efficacy issue of current BCG vaccines are the development of a subunit BCG vaccine and a recombinant BCG (rBCG) vaccine. Along with it at least 12 novel vaccines are in clinical trials [297].

The rBCG vaccines are developed by recombinantly modifying the BCG strain by insertion of Mtb antigen genes, mammalian cytokines, and adjuvants derived from bacterial toxins to induce antigen presentation leading to effective immune response generation. More than 30 recombinant BCG (rBCG) vaccines have been tested in different animal models like mice, and guinea pigs and have shown protective responses [298]. For example, a novel adjuvant Hepatitis B Virus Core VLP Particles-based vaccine has been in development which is generated by modifying the Hepatitis-B Virus core antigen by Overlap Extension PCR (OEPCR). The vaccine expresses HBc-VLP carrying a fusion VLP constructed using CFP-10 (Mtb antigen) [299].

The idea that the subunit vaccine in development can be more effective than the BCG vaccine was overshadowed by the fact that live attenuated mycobacterium was proven more effective. So, the subunit vaccine was tested to be administered as a booster to BCG. More than 26 subunit vaccines are tested in preclinical trials for their efficacy as a booster for the BCG vaccine. Some of the candidates used as boosters induced higher cytokine-producing $CD4^+$ and $CD8^+$ T cells but were unable to reduce pathogen [295,298].

In addition to these techniques, several nanoparticles (NPs) based vaccines are in development against TB. A novel mucosal intranasal TB vaccine Nano-FP1 was developed by fusion of yellow carnauba wax NPs coated with three Mtb antigen fusion proteins- Acr, Ag85B, and HBHA. A higher amount of CD4 and CD8 T cell proliferation, cytokine, and TRM production in lungs were reported post-immunization with Nano-FP1. Humoral immune responses of Ag85B-specific serum IgG and IgA were reported. The enhancement of immune response was higher in Nano-FP1 than in the BCG vaccine and it activated the APCs by IRF-3 mediated activation signal. These findings could lead to the formation of a successful vaccine against TB [300,301].

The intracellular eradication of TB can be done by NP stimulated macrophages. Pulmonary immunization of two NP-based vaccines showed effectiveness against TB. In a TB-affected mice model, the intranasal administration of liposomes consisting of PS (phosphatidylserine) and PA (phosphatidic acid) showed the pulmonary bacterial reduction up to 100-fold post 4 weeks. The outer membrane of the liposome contains the PS, and the inner membrane contains PA. An Ag85B (Antigen 85B) expressing DNA vaccine conjugated on Pluronic-stabilized sulfide nanoparticles administered to mice indicated effectiveness in reducing the bacterial load. To maintain a balance between innate and adaptive immunity the co-administration of adjuvant with a mucosal vaccination is required. The intranasal delivery of Ag85B-HBHA (heparin-binding hemagglutinin adhesion protein) by carnauba wax NPs, induced enhanced immune response in BCG primed mice [302].

Entrapment of DNA-hsp65 vaccine in cationic liposomes and its intranasal administration induced stronger Th1 immunity, reduced bacterial load, and lungs preservation in mice. The use of liposomes reduced the required amount of DNA-hsp65 vaccine up to 16 folds but managed to maintain optimum immunity. The vaccine was effective in nonhuman primates and showed a survival rate of 100% in the Cynomolgus monkey model [303]. A study conducted by [304] suggested that the DNA-hsp65 primed with BCG worked effectively. The study showed that BCG priming/DNA-HSP65 boosting intranasally induced a more effective immune response than a single dose of nasal BCG vaccine. Another DNA vaccine candidate encapsulated in chitosan NPs and coding for T cell epitopes of TB antigen ESAT-6 induced a higher T cell response than the BCG vaccine. The chitosan NPs increased immune response at the mucosal site leading to improved pulmonary immunity against TB [272].

A therapeutic vaccine developed using nano-emulsion adjuvant GLA-SE and TB antigen ID93 made it to the Phase-2a clinical trial. The phase-2a study reported that the vaccine was safe, immunogenic, and could potentially lead to improving the treatment of TB [305].

Conclusion and future outlook

For more than 200 years, conventional vaccines have contributed to the prevention of several diseases. Even if conventional vaccines were known to be effective, the emergence of epidemics and pandemics in recent years has raised concerns about their effectiveness, safety, and rapid production. Conventional vaccines have several drawbacks, but as the COVID-19 pandemic has already indicated, they have enormous potential to immunize the world. Next-generation vaccine platforms have outperformed traditional platforms in terms of immunogenicity, production, thermostability, safety, tolerability, and distribution. Nanomaterials can be functionalized with targeted molecules for specific immune responses with a flexible and rational design. Multi-directional inhibition of pathogens can be performed to combat viral diseases. Nanoparticles applied to several vaccines in clinical phage have suggested that nanoparticle-based vaccines could be a promising approach in developing antiviral agents against future viral disease outbreaks.

mRNA vaccines have the potential to prevent infectious diseases, however, the distribution is difficult owing to the structure's instability, which could be addressed by a thorough assessment. Nanocarriers have shown promising results in stabilizing mRNA for the COVID-19 vaccine formulation. The potential use of nucleic acid vaccines is emerging, as are innovative platforms such as saRNA vaccines, which require human clinical studies to demonstrate their preventive and immunotherapeutic effects. Nucleic acid vaccines can help to address the problem of cold chain distribution. A possible cancer vaccine can be developed by combining both DNA and mRNA vaccines with checkpoint inhibitor treatment. However, to improve the immunogenicity of DNA vaccines, more research is required.

More understanding of the administration route should be evaluated. Microneedle patches and other administration techniques that will enhance vaccine self-administration are being investigated. Self-administration might alleviate pressure on healthcare systems while increasing immunization rates. Multidose immunizations are costly and difficult to distribute since boosters are necessary for increased immunogenic response elicitation. As a result, single-dose immunization should receive closer attention.

Nanocarrier vaccines have the potential to generate robust immunogenicity; nevertheless, we found that several studies of peptide-based subunit vaccines are in silico, necessitating a laboratory error-free experiment to validate immunogenicity, how-

ever, this may vary by species in laboratory experiments. Immunoinformatic could be a significant tool in the development of MESV. Apart from that, the nanomaterial safety should be carefully evaluated before clinical translation. Extremely rare cases of anaphylaxis and myocarditis were reported after receiving the lipid nanoparticle-based mRNA vaccine. Anaphylaxis is suspected to be caused by lipid component polyethylene glycol-2000 in lipid nanoparticle mRNA vaccine in individuals having pre-existing anti-PEG antibodies. This phenomenon also raises safety concerns against other PEG derivatives such as sorbitol, used in other COVID-19 vaccine development [316]. The long-term humoral immunity is challenged by the lipid nanoparticle-based COVID-19 vaccine. A substantial decrease in humoral response was also observed after six months of receiving the second dose of BNT162b2. Neutralizing antibodies were substantially lower among men and immunosuppressed persons and persons 65 years of age or older [317]. To prevent increased vaccine hesitancy and promote public acceptance of novel vaccine technologies, more research into particular mechanisms such reactions, not just pertaining to PEG but also other new materials/carriers, is critically needed.

Contributors

Writing – original draft preparation, F.Z.S., D.S.; writing - review and editing, F.Z.S., D.S., P.P, A.C., A.S. A.N, S.K.S., S.K.V, P.K.P.; Figure - original draw preparation, F.Z.S.; Figure - review, P.K.P, S.K.V. S.K.S; Communication and guidance- P.K.P. All authors have read and agreed to the submission of the manuscript.

Data availability

No data was used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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