Tapping the immunological imprints to design chimeric SARS-CoV-2 vaccine for elderly population

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ABSTRACT

The impact of SARS-CoV-2 and COVID-19 disease susceptibility varies depending on the age and health status of an individual. Currently, there are more than 140 COVID-19 vaccines under development. However, the challenge will be to induce an effective immune response in the elderly population. Analysis of B cell epitopes indicates the minor role of the stalk domain of spike protein in viral neutralization due to low surface accessibility. Nevertheless, the accumulation of mutations in the receptor-binding domain (RBD) might reduce the vaccine efficacy in all age groups. We also propose the concept of chimeric vaccines based on the co-expression of SARS-CoV-2 spike and influenza hemagglutinin (HA) and matrix protein 1 (M1) proteins to generate chimeric virus-like particles (VLP). This review discusses the possible approaches by which influenza-specific memory repertoire developed during the lifetime of the elderly populations can converge to mount an effective immune response against the SARS-CoV-2 spike protein with the possibilities of designing single vaccines for COVID-19 and influenza.

Highlights

• Immunosenescence aggravates COVID-19 symptoms in elderly individuals.
• Low immunogenicity of SARS-CoV-2 vaccines in elderly population.
• Tapping the memory T and B cell repertoire in elderly can enhance vaccine efficiency.
• Chimeric vaccines can mount effective immune response against COVID-19 in elderly.
• Chimeric vaccines co-express SARS-CoV-2 spike and influenza HA and M1 proteins.

Introduction

The appearance of novel coronavirus known as SARS-CoV-2 is accountable for the disease called COVID-19 which caused more than 2 million global deaths. Coronaviruses are widespread family of viruses existing in numerous species of animals, including camels, cattle, cats, bats, and birds. Zoonotic transmission of coronaviruses seldom transpires in the human population. The previous human to human transmission of coronaviruses occurred in 2003 and 2014 with SARS-CoV and the middle-eastern and the respiratory syndrome coronavirus (MERS-CoV), respectively. Current literature describes two types of the coronaviruses alphacoronaviruses (229E and NL63) and betacoronaviruses (OC43 and HKU1) that circulates in human causing common flu [1]. The pathogenic strains that infect human are SARS-CoV-1, MERS-CoV and SARS-CoV-2, all of which belong to betacoronaviruses [2]. SARS-CoV-2 has 29.9 kb single stranded positive sense RNA genome that encodes 29 viral genes. Around 16 of these elements codes for nonstructural proteins that are copied as pair of large polypeptides (Orf1a and Orf1b) that are further processed into different polypeptides via viral proteases. Other elements codes for several viral proteins that forms variety of accessory and structural components including the spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins. The interaction between the SARS-CoV-2 spike protein and the angiotensin-converting enzyme 2 (ACE2) receptor on host cell membrane helps the virus to penetrate the cell [3]. The trimeric S protein comprises of two subunits, S1 and S2, which mediates receptor binding and membrane fusion [4,5]. Hence the spike protein has been emphasized and used as a prime candidate for vaccine development globally [6,7].
Development of vaccine is time consuming and challenging especially for the mutating RNA viruses. However, the mutation rate of SARS-CoV-2 is lower than other single stranded RNA viruses like influenza or HIV [8]. RNA viruses have the tendency to lose infectivity after acquiring mutation but few quasi-species retain infectivity and others become highly infective. These highly infective species bypass the existing immunity to establish themselves in the circulation as a new variant. The repeated viral mutation is well observed during flu season caused by influenza viruses. Every year new flu vaccine is developed based on the previous year’s epidemiological data. SARS-CoV-2 rapid multiplication and infectivity allows the virus to acquire mutations such as D164G, A222V, L18F, P681H and N501Y mutations in the Spike (S) proteins, P323L in the NSP12 protein and R203K, G204R and A220V in the nucleocapsid proteins [9]. Current epidemiological studies have identified new variants in UK (B.1.1.7), South Africa (B.1.351) and Brazil (P.1) and all three have now been detected in the USA [10]. Current vaccines under use can neutralize emerging strains but to what extent they can protect elderly population remains a major concern.

Earlier reports by CDC indicated that older adults (≥ 65 years) comprising 9% of the global population accounted for more than 80% of COVID-19 related mortality [11]. This emphasizes the severity and vulnerability of the elderly individuals to SARS-CoV-2 infection. The SARS-CoV-2 infection can overwhelmingly excite the immune system in the elderly patients thereby leading to acute respiratory distress syndrome (ARDS) which is a natural outcome of immunopathological episodes and remains a critical reason for mortality [12]. The weakened immune system of older adults is characterized by steady decline of innate and adaptive immune responses poses a major challenge for developing a vaccine that will effectively work for people of all ages [13–15]. The bottleneck to developing such vaccines is the limited knowledge about the correlates of vaccine-induced protection in older adults. Previous studies have reported various physiological and immunological changes in the human body with aging [16,17]. Therefore, vaccination does not always ensure protection to an older individual due to their inherent loss of immune functions and heightened susceptibility to infectious diseases. Thus, we must consider additional safeguards while designing a vaccine for these groups. In this review, we emphasize on the elderly population (≥ 65 years age) and their immune correlation against vaccination based on previous and current studies. We further discuss the innovative approaches of hybrid vaccines containing influenza and SARS-CoV-2 motifs to overcome the suboptimal immune response during vaccination in elderly population.

**Immunosenescence and aging aggravates COVID-19 disease**

Multifaceted alterations in immune system are the hallmark of the aging process. Immunosenescence is defined as gradual decrease in immune function with age leading to augmented susceptibility to infectious diseases [18]. Studies have reported a systemic inflammatory state with aging, known as ‘inflamming’ leading to a chronic low-grade inflammation in the lung of elderly people. The respiratory system undergoes a set of functional and structural changes with age. The progressive decline in lung functions including anatomical changes of the thoracic cage leading reduction in chest wall compliance, decreased strength of respiratory muscle affecting airway clearance are observed with aging [19,20]. Uncontrolled inflammation may impair lung, heart and kidney functions due to impeded resolution and presence of frailty in the older individuals [21]. Older individual has dysregulated innate immune cells lacking efficient phagocytosis and egress mechanism from the site of infection which further increases proinflammatory response by engaging with pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) resulting in further tissue damage [22,23]. Innate immune response is primarily responsible for initial inflammations leading to the accumulation of phagocytes (neutrophils and macrophages) at the site of infection. The phagocytes clear-up the virus infected cells and dead or apoptotic cells damaged due to viral multiplication [24,25]. Alveolar macrophages (Aφs) provide the first line defense against respiratory pathogen and plays a pivotal role maintaining tissue homeostasis [26]. In a resting state, Aφs binds to the alveolar type II epithelium and expresses regulatory ligands (CD200 and TGFβ) and anti-inflammatory cytokines, such as IL10 and TGF β [27,28]. Upon sensing any danger signals or insult, Aφs undergo molecular reprogramming to regulate a complex interaction between suppressive and activating signals [29,30]. Neutrophils from aged animals also show a similar reduced ability to mount an innate immune response like formation of neutrophil extracellular traps (NETs) [31,32]. Lower expression of Atg5 in aged mice leads to reduced autophagy mediated NET formation [33]. Innate immune responses promote adaptive immunity by
stimulating the antigen presenting cells (APCs) to capture and present antigen and by producing cytokines, chemokines and co stimulatory molecules that is required for optimum T helper cell response at the secondary lymphoid organs [34]. Antibodies and CD4+ T cells are essential to neutralize virus particles by opsonization and killing of virus infected cells by cytotoxic T cells [35,36]. Aging significantly influences thymic involution with a gradual declination of the structural integrity of the thymus in aged people resulting in a steady decrease in the T cell production [37]. Older individuals have reduced number of naïve T helper cell populations and distorted lymph node architecture critical to retain naïve cells which promote germinal center reaction after activation by APCs to produce affinity matured and class switched antibodies (Figure 1A) [38,39].

Outcome of any infectious disease depends on both the arm of the immune responses. Aging leads to gradual changes in every aspect of host immunity affecting both the innate and adaptive immune arm resulting in heightened inflammatory response or cytokines storms during robust viral replication and cell damage that defines the disease pathophysiology of COVID-19 [40,41]. Recent clinical studies have reported significant upregulation in proinflammatory cytokines (TNF-α, IL-1β, IL-6, IL-8, G-CSF and GM-CSF) and chemokines such as MCP1, IP10 and MIP1α in severely affected COVID-19 individuals [42–44]. Cytokines storms are characteristics for many viral diseases like SARS, MERS and H5N1 infection including the current COVID-19, providing them an inflammatory disease signature [45,46]. High SARS-CoV-2 replication in the type 2 pneumocytes causes it to differentiate from a quiescent state to a highly proliferating inflammatory state resulting in an impairment of suppressing signals [47].

Severely affected SARS-CoV-2 patients of all ages have very low number of T regulatory (Tregs) cells [48]. Patients severely suffering from COVID-19 were reported to have an uncontrolled inflammation associated with pneumonia, myocarditis and microvascular

**Figure 1.** Factors influencing COVID-19 outcomes in the elderly population. A. Immunosenesence mediated changes in innate and adaptive immune response might affect the COVID19 disease outcome. The immunosenesence is characterized by thymic involution, modified T and B cell responses due to alteration in the naïve/memory lymphocyte population, and heightened serum levels of IgG and IgA with a lower level of IgM and IgD, and a weak response to newly encountered pathogens/antigens such as SARS-CoV2 or influenza or after vaccination which may lead to severe disease outcome specially within the elderly population. B. Both the humoral (mediated by SARS-CoV-2 specific neutralizing antibodies) and cellular immune response (mediated by CD8+T cells that kills virus infected cells) are indispensable for effective COVID-19 immune response. Diverse memory T and B cells in elderly individual might act as a reservoir that can be tapped to raise a strong adaptive immune response against SARS-CoV-2 by tweaking novel vaccine design.
thrombosis, and cytokine storms which can be effectively dealt with increased Tregs [49]. Several clinical studies with SARS-CoV-2 patients have reported the presence of proinflammatory cytokines including IL6, IL8 and IL12, IFN γ and IL-2 [50,51]. ACE2, the key receptor of SARS-CoV-2, is reported to be downregulated after SARS-CoV-2 infection, thereby leading to ARDS and cardiac injury due to increase accumulation of angiotensin II [52,53].

**COVID-19 vaccination and immune response within the elderly population (≤65 years)**

All the COVID-19 vaccines are designed to induce an effective antiviral response and the efficacy is analyzed by measuring the neutralizing antibody titer against the spike protein or receptors binding domain of spike [54]. The total number of CD4+ and CD8+ T cells were dramatically reduced in COVID-19 patients, especially among elderly and ICU patients. Moreover, T cells in these individuals shows high expression of PD-1 and Tim-3 expression compared to healthy controls, an indicative of T cell exhaustion [55]. A recent study compared T cell memory response in COVID-19 recovered patients (28 with mild disease and 14 with severe disease) and indicated that mild cases have higher proportions of SARS-CoV-2 specific CD8+ T cells whereas individual with severe COVID-19 have more SARS-CoV-2 specific antibody titer compared to mild case [48]. These finding suggest that humoral immune responses alone were unable to protect an individual from severity of the disease. Earlier study by Wang et al, have found that H7N9 infected influenza patients discharged within 2–3 weeks have early prominent H7N9-specific CD8+ T cell responses, while individuals with prolonged hospital stays have late recruitment of CD8+ or CD4+ T cells and antibodies simultaneously [56]. These studies suggest that recovery phases of acute viral infection are dependent on effective CD8+ T cell response. All these observations suggest an optimum T cell response is indispensable for the resolution of COVID-19 disease which may be lacking in elderly population and provide rationalization for vulnerability toward severe SARS-CoV-2 infection (Figure 1B). The use of spike as a vaccine candidate can be effective to induce effective CD8+ T cell responses, as new findings also suggest that during natural infection a significant number of CD8+ T cell responses were observed with spike derived peptide pool during ex vivo challenge [48,56,57].

Inovio pharmaceuticals had developed INO-4800, a novel DNA based vaccine candidate which is administered directly into the skin of the recipient through Cellectra 2000 [58,59]. As soon as the genetic sequence of the novel corona virus was published [60], Inovio constructed a DNA based vaccine without further delay utilizing their proprietary DNA medicine platform technology. It successfully prompted antigen specific robust T cell mediated immune response and neutralizing antibody production that effectively blocked the binding affinity of the spike protein to ACE2 receptor. Phase 1 clinical trial with INO-4800 exhibited excellent safety and tolerability and remained immunogenic in 100% (38/38) of the vaccinated individuals by evoking either or both humoral or cellular immune responses [59]. Currently a study with populations 51 years and older is underway (NCT04336410).

The mRNA vaccines are similar to DNA vaccines off late gaining much required attention due to their robust immunogenicity, easy production scale up, and the development of lipid nanoparticles for efficient delivery [61]. With this new technology viral antigen can be delivered as mRNA sequence within lipid nanoparticles (LNP) instead of viral protein molecules [62–64]. The antigen is then expressed in the recipient cells of the vaccinated individual to mount humoral and cell mediated immune responses (Figure 2). There are several mRNA vaccines that are under development or in trials for COVID-19 (Table 1).

Moderna in collaboration with the National Institute of Allergy and Infectious Diseases (NIAID) have developed a LNP encapsulated mRNA vaccine named mRNA-1273 that encodes full-length perfusion stabilized spike protein [65]. The perfusion form encompasses a transmembrane anchor and an unaltered S1-S2 cleavage site. The substitution mutation of two proline residues in the S2 region stabilizes the newly designed protein [66]. The genetic similarity of the MERS-CoV and SARS-CoV-2 lead to the development of a single synthetic viral RNA molecule as a vaccine candidate making it safer for human use. The phase 1 and phase 2 clinical trials were carried out to evaluate the safety, reactogenicity, and immunogenicity of the vaccine in human. Antigen-specific neutralizing antibody developed in a *Rhesus macaque* model also enhanced protection in the lung without any symptoms of pathological changes [67]. The participants in this trial were between the age group of 18–70 years. Vaccine trials have found mild adverse events of fatigue, chills, headache, and pain at the site of the injection area were observed. This study also reported the presence of neutralizing antibodies in the serum with strong CD4+ mediated T cell responses. The phase III trial had 25.3% population with 65 years and older age. The vaccine evoked
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...compared to those in the placebo group during phase III trial, usually enduring a few days. The reactogenicity were commonly mild to moderate and generally less frequent in older adults (≥65 years) as compared to younger participants [68].

In USA, the BNT162 mRNA vaccine developed by BioNTech and Pfizer, encodes an optimized SARS-CoV-2 receptor-binding domain (RBD) antigen which is currently used for vaccination [69]. A prime-boost regimen strategy was explored with this vaccine and reported to be well tolerated during the early-stage human trial and elicited dose-dependent immunogenicity [70]. During December 2020, monitoring by the Vaccine Adverse Event Reporting System identified 21 cases of anaphylaxis (which is a severe,
life-threatening allergic reaction that occurs rarely after vaccination) following administration of a reported 1,893,360 primary doses of the Pfizer-BioNTech COVID-19 vaccine (11.1 cases per million doses); 71% of these appeared within 15 minutes of vaccination. Recent reports of 23 elderly patients death in Norway after having regular doses of Pfizer/BioNTech mRNA vaccines created panic in certain parts of the world about their safety and tolerability [71]. The higher dose of conventional mRNA vaccine can induce an adverse side effect in the vulnerable frail group. Health authorities are investigating the reason behind such deaths in the elderly population [71]. Both Moderna and Pfizer/BioNTech vaccines require two doses and the reactogenicity is typically higher after the booster dose.

Novavax investigated their proposed vaccine, NVX-CoV2373, full length S subunit protein, with or without Matrix-M™ as an adjuvant. The anti-Spike IgG antibody was found to be present in the all-vaccine recipient right after the single dose and the presence of the adjuvant further augmented the polyfunctional CD4+ T cell responses [72]. In this study participants were between 18 to 59 years of age were divided in to multiple groups to carry out the immunogenicity of the vaccine. The phase 3 trial aimed in enrolling 30,000 people in the United States and Mexico with two cohorts: individuals 18 through 64 years old and those ≥ 65 years older, with a goal of enrolling at least 25% of all volunteers who are.

Live attenuated vaccines are produced by creating a genetically weakened variant of the virus that replicates to a constrained extent, causing no or mild disease but induces immune responses comparable to that induced by natural infection [73]. Codagenix, Inc. in collaboration with Serum Institute of India had developed CDX-005, an engineered live attenuated virus with the help of codon deoptimization software platform [74]. Although the amino acid sequence of the constructed mutated whole virus particle is structurally identical to the wild type SARS-CoV2, it is unable to replicate in the host. The newly designed nonpathogenic attenuated virus reported to generate strong humoral and T cell mediated immune responses in the preclinical studies with mice and guinea pigs and with non-human primates.

The ChAdOx1 nCoV-19 vaccine developed by the University of Oxford in collaboration with AstraZeneca and the Serum Institute of India, expresses a full-length, wild-type version of the Spike protein. The ChAdOx1 nCoV-19 vaccines are typically based on a modified adenovirus that has been engineered to express the Spike protein and has been disabled from replication in vivo by the deletion of parts of its genome. During safety and immunogenicity study with ChAdOx1 nCoV-19 the local and systemic reactions including pain, feeling feverish, colds, muscle ache, headache, and malaise were more frequent in the ChAdOx1 nCoV-19 groups which were lessened by the use of prophylactic paracetamol [75]. A recent study was conducted from four ongoing blinded, randomized, controlled trials done across the UK, Brazil, and South Africa. In this trial, in Britain, the registered population was predominantly white and, in younger age groups, incorporated more female participants due to the focus on recruitment of health-care workers whereas in Brazil, there was a larger proportion of nonwhite ethnicities. Study participants aged 18 years and older were randomly assigned to ChAdOx1 nCoV-19 vaccine or control. Overall vaccine efficacy across both groups was around 70-4% [76]. The phase 1/2 trial proposes two-dose regime where the booster dose induces a strong T cell response and anti-spike neutralizing antibody production and the vaccine is currently under phase 3 trial [77]. Recently many European countries halted the use of AstraZeneca’s vaccine in March 2021 following the report of symptoms that led to at least 15 deaths. However, vaccination was resumed on European Medicines Agency (EMA) recommendation stating the benefits of vaccine outweighs its risks [78].

All these vaccines use various advanced technologies and are reported to mount a protective immune response by generating antigen specific neutralizing antibodies and an optimum T cell response. Most effective COVID-19 vaccine must induce an effective CD8+ T cell response with neutralizing antibody titer. However, current determinant of vaccine efficacy doesn’t provide enough importance to CD8+ T cell response. Also, it is not known how long vaccine induced neutralizing antibody titer will provide protection from infection or reinfection. Current COVID-19 vaccine development must include the capability to efficiently induce CD8+ T cell memory response that will provide an additional layer of protection to avoid severe consequences during future reinfecction. Vaccines developed using the whole virus not only induced CD8+ T cell response against Spike but also for other viral proteins like M, Nucleoprotein ORF3a, ORF6 and ORF7a [57,79–81]. Therefore, spike-based vaccines lack diversified CD8+ T cell responses, limiting the breadth of immune response which may play important role during resolution phase of the infection. Thus, all the next gen mRNA-based vaccines have an inherent drawback of lower capacity to induce diverse immune response particularly within
elderly individuals with low antigens specific naïve CD4+ or CD8+ T cells [82]. Although the current phase 3 trial results of mRNA-1273 vaccine suggests that it is safe and effective at preventing symptomatic COVID-19 in adults but its efficacy within elderly population (≥ 65 years) remains matter of concern. Recently, BioNTech and Pfizer have also expanded this study to determine vaccine efficacy within the elderly group but the data is yet to be public (NCT04368728) [70]. Gradual mutations in spike protein will make the current vaccines no longer effective similar to flu vaccines. However live attenuated SARS-CoV-2 strains can overcome such challenges by mounting an effective CD8+ T cell response to various relatively conserved proteins.

**Lessons learned from earlier vaccination strategies for elderly population**

New vaccines are being developed by modifying the antigen, the route of administration, higher dose administration and implementing adjuvants. Administering higher dose via parenteral route activates follicular DCs and subsequently antigen presentation and B cell activation in the older population [83]. Majority of the commercialized licensed vaccines imparts reduced immunogenicity and lesser protective efficacy among elderly population due to evident decrease in naïve T cell population, distorted lymphoid architecture whereas polyfunctional CD8+ T cell remain unaffected with aging [84]. The commercially available widely used vaccines for elderly people especially in the developing countries have recommended guidelines for vaccination in elderly people against infections like influenza, pneumonia, herpes zoster, and tetanus (Table 2). Waning immune system generates a weaker immune response toward vaccination in elderly population and requires repeated boosters.

Adjuvants are recognized to enhance the potency and endurance of specific immune responses generated to the administered antigen. However, live attenuated bacteria or viruses do not always require any adjuvants due to their structural components or genetic material that can induce the host's innate immune system. One such licensed attenuated vaccine, Zostavax, was reported to be 33% protective but can effectively reduce hospitalization suffering from herpes zoster and postherpetic neuralgia among the elderly [85], whereas TIV (trivalent inactivated influenza vaccine) contains two antigens from influenza A (H1N1, H3N2 strain) and one from influenza B strain (Victoria or Yamagata) along with MF59, is an oil in water emulsion based adjuvant composed of squalene, tween 20 and span 85. A cumulative effect of all of the components of MF59 was reported to impart enhanced immunogenicity by activating the genes supporting trans-endothelial cell migration, favoring antigen uptake and its transportation to the lymph node [86,87]. Matrix-MTM and ASO3 are also reported to enhance the antigen uptake and favor the augmentation of Th1 and Th2 responses which will be beneficial for elderly group as they have dysregulated T cell machinery [88]. ASO3 is an immuno-enhancing oil in water emulsion-based adjuvant, comprising of squalene, polysorbate 80 and α-tocopherol, used in the Influenza (H1N1/pdm2009) vaccine formulation [89]. It was reported to generate microneutralizing antibodies which can persist up to more than a year after immunization with a much lower dose of antigen whereas other non-adjuvanted vaccine required higher dose of antigen but showed a decline in antibody responses over this time [90]. Another liposome-based adjuvant, ASO1, comprises MPL (Lipid molecule named 3-O-desacyl-40-monophosphoryl lipid A obtained from Salmonella minnesota) and QS-21 (a lytic saponin fraction of QuilA) was used to enhance vaccine efficacy of a subunit zoster vaccine named Shingrix [78]. A significant increase in humoral as well as cellular immunity including cytotoxic T cell responses with a vaccine efficacy of more than 90% was observed compared to non-adjuvanted vaccine [91]. Influenza virosomes have also been used as an adjuvant and carrier system owing to its excellent antigen delivery mechanism to the target sites [92]. Structurally the modified virus is composed of unilamellar phospholipid membrane incorporating surface hemagglutinin (HA) and neuraminidase (NA) antigen but lacks the viral inner core and genetic information making it replication deficient. Mimicking the natural infection, virosome adjuvanted influenza vaccine effectively induced humoral antibody and cytotoxic T cell activity in elderly people [93,94]. Microparticles of β-d-[2'-1] poly(fructo-furanosyl)α-d-glucose (delta inulin) proved to be safe and nontoxic adjuvant for human use. It skewed immune responses to the Th1 or Th2 depending upon the type of antigen it is administered with, functioning as an amplifier. It is effectively used in Hepatitis B, influenza, and HIV [95–97]. The most extensively used adjuvant is Matrix-M, comprises cholesterol, phospholipids and saponin, augments humoral and cellular immune responses. Although, the mechanism underlying the potency of this adjuvant is not yet resolved but it creates a milieu of activated T and B lymphocytes, NK cells, neutrophils and monocytes at local lymph nodes [98,99]. Researchers had employed vector-based
vaccines with influenza nucleoprotein and matrix protein and reported to mount a robust immune response that compensates for annual influenza vaccination [100]. Non replicating viral vectors are also used to deliver potential vaccine candidate. Modified vaccinia Ankara viral vector containing five respiratory syncytial virus (RSV) proteins were being tested in a clinical trial and reported to induce Th1 type cell mediated immune responses among older adults [101,102].

A 23 valent polysaccharide based pneumococcal vaccine, containing a T cell independent antigen, elicits exclusively humoral immunity but fails to generate immunologic memory owing to its lack of direct recognition by T cells [103]. Nonetheless, chemical conjugation of polysaccharide to a carrier protein makes 13 valent conjugate pneumococcal vaccine into a T cell dependent antigen which eventually generates high affinity matured antibody and immunological memory [104]. A prime-boost regimen with Prevnar13 followed by Pneumovac23 further augmented the immune responses among elderly. This prime-boost strategy resulted in improved serotype specific IgG responses [105]. Much deeper perceptive is required to develop potential vaccine candidate for the elderly people to boost their immune system.
In addition to the appropriate selection of adjuvants and suitable modification of antigens, vaccine efficacy largely depends on several host factors. Considering the weaken immune system of the elderly people and several other co-morbid clinical conditions, effective vaccination must overcome these hurdles successfully. An aged immune system harbors multitude of changes especially a steady decline in naïve CD4+ T cell number due to inability of lymphocyte generation in the primary lymphoid organ. Parallelly, alternation of secondary lymphoid structure and function results in altered T cell trafficking and a restricted T cell repertoire [16,106]. Depletion of naïve CD4+ T cells during vaccination in elderly people significantly reduces the vaccine efficacy. The influenza specific memory T cells are directed toward conserved protein sequences across numerous influenza strains available. Therefore, these memory T cells can successfully confer cross protection against numerous strains of influenza and strategically bestows an alternative approach to treat elderly people with an added advantage. Older individuals have encountered several influenza strains throughout their life span and unknowingly carries diverse influenza specific memory CD4+ T cell populations [107].

**SARS-CoV-2 mutations and vaccine effectiveness**

Researchers around the world are toiling to develop targeted vaccines against COVID-19 in record time. The spike glycoprotein present on the surface of the coronavirus that helps in receptor binding and membrane fusion was proposed to be a potential vaccine candidate by various research groups [108]. The viral S protein consists of two subunits: S1 and S2. The S1 domain contains the receptor-binding domain (RBD) crucial for the initial attachment of the virus to the host cell, and S2 prompted viral fusion with the host cells to initiate the infection [109,110]. Numerous studies have identified the probable B cell epitopes from the spike protein which often acquires point mutations [111–114]. Further studies also indicate that the variable region is more prone to point mutations than the stalk region [115,116]. So, the efficacy of the spike-based vaccines can be compromised by acquired mutations over time. Numerous naturally occurring spike variants like Q414E, N439K, G446V, K488N, I472V, A475V, T478I, V483I, F490L, H519P, D614G and Q321L have been reported to decrease the sensitivity to neutralizing antibody and convalescent sera [117]. Most of these naturally occurring mutations found in RBD domain of variable region of spike protein. Our analysis of B cell epitopes and surface accessibility of spike protein, S1 region (S1: aa13 to aa685 and RBD: aa319 to aa514) and stalk domain (S2: aa686 to aa1273) shows that stalk directed B cells epitopes are less accessible (Figure 3). But accumulation of RBD mutations may influence vaccine effectiveness both in general as well as in the elderly populations. Consistent with our findings the RBD of spike is a target for 90% of the neutralizing SARS-CoV-2 immune sera indicating their immunodominance and the less accessible stalk B cell epitopes probably plays minor role in viral neutralization [113]. The E484K a receptor-binding-domain mutation, and S01V2 mutation N501Y was stated to be linked with escape mutant from neutralizing antibodies [118,119]. The current BNT162b2 vaccine elicited sera can neutralize N501Y variants [120]. However, convalescent sera from COVID-19 individual were unable to neutralize a novel bat coronavirus (W1V1-CoV) that is highly homologous to SARS-CoV-2 and uses ACE2 receptor for host cell entry indicating their pandemic potential in the future [121,122]. Immune selection of antigenic variants seldom occurs within immune-compromised or elderly populations due to their inadequate immune response or sustained pathogen presence [123]. Altogether these findings indicate that humoral response against spike-based vaccines may reduce their efficacy with the emergence of new antigenic variants inadequate immune response within the elderly population may facilitate such antigenic variant selection.

**Strategies to induce SARS-CoV-2 specific immune response within elderly population**

Researchers discovered constant regions of influenza virus trimeric HA especially in the stalk domain and utilized it to generate neutralizing antibodies which will impart universal protection against several influenza strains [124–126]. Analyzing and identifying the conserved region of the spike protein of SARS-CoV-2 can lead to generation of a hybrid or chimeric vaccine candidate harboring both the influenza and SARS-CoV-2 antigens part is possible. Our Vaxijen based prediction shows the presence of some B cell epitopes in the stalk region but due to their low surface accessibility stalk region, unlike influenza HA stalk may not be an appropriate vaccine candidate (Figure 3). A hybrid/chimeric protein can be generated by combining the HA and spike protein as the formation of the trimeric assembly largely depends on the residues present in the stalk region. The residues from the stalk region form numerous H-bond
interaction to form the trimeric assembly of the Spike protein and the residues from the variable region also gets stabilized by interacting with the stalk region (Figure 4A) [109,127]. The baculovirus insect cell expression system can be used to produce hybrid and/or chimeric VLPs comprising the SARS-COV-2 spike protein, stalk region of HA and the influenza M1 protein (Figure 4B) [128,129]. Chimeric protein with intact stalk region for the HA protein and the Spike variable or RBD domain will allow utilization of influenza specific CD4+ T cell populations to mount an effective antibody response in germinal center reactions. Each VLP will have both Spike, HA and M1 capable of utilizing corresponding CD4+ T cells help during germinal center reaction with spike specific B cells subsequently promoting affinity maturation and class switching (Figure 4C). This influenza specific CD4+ T cells can interact with spike specific B cells when the same B cell clone portrays MHC II containing influenza specific peptides for T cell help during germinal center reaction in the secondary lymph node. This may overcome the lack of spike

**Figure 4.** Structure and design of hybrid or chimeric VLP for SARS-CoV-2 vaccination. (A) The trimeric assembly of SARS-COV-2 Spike protein and Influenza HA. Residues from stalk region represented in a cartoon format, different colors signifies three different chains. The residues from the variable region those are making H-bond with the stalk are labeled (representative from a single chain) whereas all the other residues shown as a ribbon diagramme. Upper figure showing the H-bond interaction profile for a single chain stalk region (pink) within the trimeric assembly. (B) Influenza Hemagglutinin (HA) and SARS-CoV-2 Spike contain head and stalk domain. Matrix protein 1 (M1), either with chimeric Spike (stalk from HA with head from Spike), (C) Schematic diagram showing cooperation between influenza induced memory T cells and development of spike specific immune response.
antigen specific naïve or matured CD4+ T cells population which lack in aged individual due to age related defects. This strategy was explored widely against polysaccharide vaccines, where polysaccharide is attached directly to a protein molecule to mount strong T cell dependent immune responses to produce high affinity and class switched antibodies. The repetitive antigenic structure present on the surface of the VLPs makes it easier to be recognized and prompted a strong induction of humoral and cell mediated immune responses.

This novel approach of designing of chimeric or hybrid vaccine will give additional protection against influenza viruses responsible for thousands of deaths every year during seasonal flu. Recently phase 1 clinical trials were conducted with chimeric HA (consisting of constant stalk region with variable head domain of different strain of influenza viruses) inducing broadly protective HA stalk directed antibodies in vaccinated individuals [125]. Natural head domain of HA replaced with spike head can induce spike specific antibodies as well as antibodies directed against the HA stalk. This will further boost influenza specific immunity within vaccinated individual.

**Conclusions**

The terrific brunt of SARS-CoV-2 infection and the scarcity of available effective treatments specially to cure elderly individuals require more rational approach. Multifactorial changes in developing an appropriate cell mediated immune responses in elderly individual is of paramount need for designing an efficacious vaccine. The vaccines from Moderna, Pfizer and AstraZeneca, which are under emergency use in the USA and Europe will protect an individual from COVID-19 but the durability within normal or elderly population is unknown. The disease manifestation of COVID-19 has similarities with seasonal flu caused by influenza viruses. The ultimate goal is to design a universal vaccine for influenza, SARS-CoV-2 or other coronaviruses. In this article we highlighted the design of vaccine molecule having multiple antigens from different viruses can complement each other to mount an effective immune response especially in the elderly population with low naïve immune cell population. Stalk region of HA is relatively conserved and stalk directed antibodies can give protection to wide range of influenza strains. Therefore, chimeric vaccines for both influenza and SARS-CoV-2 will be a significant boost to deal global challenges against seasonal or pandemic flu viruses. Researchers should move forward with the idea of making a single chimeric molecule as vaccine candidates for both influenza and SARS-CoV-2. The idea of making chimeric molecules can be incorporated with mRNA, DNA, and adenovirus-based vaccine delivery system. This will have a significant impact on worldwide vaccination drive and their economic impact in the future to save human lives.

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