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REVIEW ARTICLE

Immunology to Immunotherapeutics of SARS-CoV-2: Identification of Immunogenic Epitopes for Vaccine Development

Apoorva Pandey[1] · Riya Madan[2] · Swati Singh[3]

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Abstract

The emergence of COVID19 pandemic caused by SARS-CoV-2 virus has created a global public health and socio-economic crisis. Immunoinformatics-based approaches to investigate the potential antigens is the fastest way to move towards a multiepitope-based vaccine development. This review encompasses the underlying mechanisms of pathogenesis, innate and adaptive immune signaling along with evasion pathways of SARS-CoV-2. Furthermore, it compiles the promiscuous peptides from in silico studies which are subjected to prediction of cytokine milieu using web-based servers. Out of the 434 peptides retrieved from all studies, we have identified 33 most promising T cell vaccine candidates. This review presents a list of the most potential epitopes from several proteins of the virus based on their immunogenicity, homology, conservancy and population coverage studies. These epitopes can form a basis of second generation of vaccine development as the first generation vaccines in various stages of trials mostly focus only on Spike protein. We therefore, propose them as most potential candidates which can be taken up immediately for confirmation by experimental studies.

Abbreviations

APCs	Antigen presenting cells
ARDS	Acute respiratory distress syndrome
COVID-19	Coronavirus disease 19
CoVs	Coronaviruses
DC	Dendritic cell
DLNs	Draining lymph nodes
dsRNA	Double-stranded RNA
hACE2	Human angiotensin-converting enzyme 2
HLA	Human leukocyte antigen
IFN	Interferon
IKKε	Inhibitor of NF-kappa B kinase epsilon
IRF3	IFN regulatory factor 3
MAVS	Mitochondrial antiviral signaling protein
MDA5	Melanoma differentiation-associated protein 5
MHC	Major histocompatibility complex
MyD88	Myeloid differentiation primary-response protein 88
nsp	Nonstructural proteins
PAMPs	Pathogen-associated molecular patterns
PRRs	Pattern recognition receptors
RBD	Receptor-binding domain
SARS-CoV-2	Severe acute respiratory syndrome coronavirus-2
ssRNA	Single-stranded RNA
TLR	Toll-like receptors
TMPRSS-2	Transmembrane protease serine protease-2
TNF-α	Tumor necrosis factor alpha
TRAF3	TNF receptor-associated factor 3
TRIF	TIR domain-containing adapter protein inducing IFN-beta

Apoorva Pandey and Swati Singh are joint first author.

✉ Swati Singh
singhswati.mh@gmail.com

Apoorva Pandey
apoorvap1908@gmail.com

Riya Madan
6madanriya8@gmail.com

[1] Indian Council of Medical Research, V. Ramalingaswami Bhawan, Ansari Nagar, P.O. Box No. 4911, New Delhi 110029, India

[2] Indian Institute of Science Education and Research (IISER) Mohali, Knowledge City, Sector 81, Sahibzada Ajit Singh Nagar, Punjab 140306, India

[3] Department of Zoology, University of Delhi, Delhi 110007, India

Introduction

In December 2019, a cluster of pneumonia cases were reported in Wuhan, China. The cases were attributed to an unidentified coronavirus species, later termed as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) due to its similarity with SARS-CoV and the disease was called as coronavirus disease 19 (COVID-19). On March 11, it was declared a pandemic by WHO. As of July 8, 2022, the total number of confirmed cases are over 546 million and death toll due to this catastrophic disease has reached a whopping 6.3 million globally [1].

SARS-CoV-2 virus primarily affects the respiratory system through direct or indirect respiratory exposure. COVID-19 primarily attacks the lungs, but is reported to also affect other organs such as heart, kidney, ileum, and urinary bladder [2]. The primary mode of infection is human-to-human transmission through close contact via inhaled droplets and aerosols generated during cough or sneeze of an infected individual and/or via fomites. It has proved to be detrimental with major health concerns in the young, elderly, and immunocompromised individuals, as it may lead to exacerbation of pre-existing conditions [3]. The virus targets the respiratory epithelial lining with a gamut of clinical manifestations from asymptomatic, quasi-symptomatic to severe end-stage lung disease [4]. The most commonly reported symptoms include fever, nonproductive cough, dyspnea, myalgia, fatigue, dysgeusia, vomiting, diarrhea, decreased leukocyte counts, and pneumonia [5]. The disease severity is likely to be a combination of direct virus-induced pathology and the host inflammatory response to infection.

The probable incubation period in SARS-CoV-2 varies between 2 and 14 days during which the virus can be transmitted [6]. Its rapid spread occurs with a basic reproduction number (R_O) of 2.2–2.6, which implies that on an average each individual has the potential to spread the infection to 2.2 other people [7].

Coronaviruses (CoVs) belong to the family Coronaviridae which is largely divided into four genera; α (HCoV-229E and NL63), β (highly pathogenic—MERS-CoV, SARS-CoV, and low pathogenic-HCoV-OC43 and HCoV-HKU1), γ, and δ based on their genomic structure [8]. Of these, HCoV-229E and HCoV-NL63 are known to cause common cold. α and β CoVs are known to infect mammals [8] while γ and δ CoVs can infect both birds and mammals. Coronaviruses are highly prevalent and widely distributed due to efficacious host-switching owing to extensive animal reservoirs especially bats and rapidly increasing human–animal interface activities, frequent genome recombination and plasticity of their receptors. Therefore, novel coronaviruses are likely to emerge episodically in humans owing to frequent cross-species infections and occasional spill over events [9].

The early genome sequencing performed on viruses isolated from patients revealed SARS-CoV-2 as a member of the genus *Betacoronavirus* and the subgenus *Sabecovirus*. The whole genome sequence of SARS-CoV-2 after the Blastn search has revealed that it has ~79% similarity with SARS-CoV (a member of subgenus *Sabecovirus*) and ~50% similarity with MERS-CoV (subgenus: *Merbecovirus*) [10, 11].

Genome sequencing performed on viruses isolated from patients revealed that SARS-CoV-2 has ~79% similarity with SARS-CoV and ~50% similarity with MERS-CoV [12]. SARS-CoV-2, like SARS and MERS CoVs, is the third zoonotic virus known to cross the species barrier. The virus RaTG13, identified from bat species *Rhinolophus affinis* sampled in caves of Yunnan province in 2013 is the most closely related virus to SARS-CoV-2 [13]. Spike protein of the virus shares 76% and 97% of amino acid similarity with SARS-CoV and RaTG13, respectively, while receptor-binding domain (RBD) shares 74% and 90.1% homology with SARS-CoV and RaTG13, respectively [14], suggesting that bats play a key role as coronavirus reservoirs [13]. However, the possibility and existence of its intermediate host is still unknown.

Coronaviruses are enveloped, positive-sense, single-stranded RNA (ssRNA) viruses with genome size of 26–30 kb [15] encoding structural and nonstructural proteins. The structural proteins include; Spike (S), Envelope (E), Membrane (M), and Nucleocapsid (N) [16]. Spike is a transmembrane trimeric glycoprotein protruding from the viral surface which determines the diversity of coronaviruses and host tropism. It contains an RBD which attaches itself to the host cell during its entry. Each monomer of trimeric S protein comprises of 2 functional units; S1 responsible for binding to the host cell receptor and S2 subunit for the fusion of the viral and cellular membranes. The genome of SARS-CoV-2 consists of at least ten open reading frames (ORFs). The ORF1a/b, spanning about two-thirds of viral RNA, is translated into two large polyproteins. In SARS-CoV and MERS-CoV, these two polyproteins, pp1a and pp1ab, are processed into 16 nonstructural proteins (nsp1-nsp16) that form the viral replicase transcriptase complex [17].

Since the time COVID-19 was declared as a pandemic, numerous SARS-CoV-2 variants have emerged resulting in new waves of infections. Genomic surveillance, owing to over a million of genome sequences deposited in Global Initiative on Sharing All Influenza Data (GISAID), has accelerated to monitor the virus evolution and evaluate the similarities between the globally circulating variants with the vaccine strains. Phylogenetic analysis of GISAID sequences has highlighted multiple clades on the basis of common mutations. The reference strain belongs to the L clade and rest all have been clustered into: S (L84S in NS8), V (L37F and G251V in NSP6 and NS3), G (D614G

in spike protein), GH (NS3-Q57H), GR (N-G204R), GV (S-A222V) [18]. Those, which do not belong to any of the above mentioned, are designated the 'O' clade. Efforts have also been made in the direction of clustering large datasets of SARS-CoV-2 genomic sequences more efficiently and at a faster rate [19]. Certain computational studies have also demonstrated the robust methods to predict the clade and VOCs emergence [20, 21].

Recently, Zhou et al. [13] reported that like SARS-CoV, SARS-CoV-2 uses human angiotensin-converting enzyme 2 (hACE2) as one of the cell surface receptors. ACE2 receptor is seen to be highly expressed in nasal epithelial cells, goblet/secretory cells and ciliated cells throughout the respiratory tract [22]. In lungs, it is highly expressed in respiratory and vascular endothelium, alveolar monocytes, macrophages and type I and II alveolar epithelial cells [12]. ACE2 expression is also widely present in endothelial cells of small and large arteries and veins in other organs, such as heart, ileum, kidney and bladder [23]. Current observations indicate CoVs being particularly adept at evading host immunity at the early stage of infection leading to dampening of immune responses. This partly explains why they tend to have a longer incubation period, as compared to influenza (1–4 days) [24]. As the details of the cellular responses to SARS-CoV-2 virus are not well established, a likely course of events can be postulated based on the past studies with SARS-CoV and MERS-CoV.

The COVID-19 pandemic represents one of the greatest health emergencies since the influenza outbreak of 1918, providing an unprecedented challenge for prophylactics and development of effective therapeutics. As developing an effective vaccine becomes a prime concern, immunoinformatics methods to identify potential vaccine candidates offer a rapid and promising approach in the absence of experimental data, reducing both time and cost significantly. This review abridges the immunology of SARS-CoV-2 and explores the potential of B and T cell epitopes as promising immunogenic candidates for development of vaccine. The present study reviews the available literature on in silico vaccine candidature studies for SARS-CoV-2 and shortlists the most potential vaccine candidates. In the wake of importance of T cell response to SARS-CoV-2, we further investigate their capability to induce either a protective Th1 response or immune-suppressive Th2 response using online servers of cytokine prediction.

Pathogenesis of COVID-19

Upon binding to the host receptors, the virus gains entry through endocytosis or membrane fusion. As the viral contents are released inside the host cells, replication and biosynthesis of viral proteins occur which is ensued by assembly and release of new particles [13]. Coronavirus S protein has been reported as a significant determinant of virus entry into host cells [25]. Spike glycoprotein binds to its cellular receptor, ACE2 and a C-type lectin, also called L-SIGN (CD209L) in SARS-CoV [26, 27], ACE2 in case of SARS-CoV-2 [28], and Dipeptidyl-peptidase 4 (DPP4) in MERS-CoV [29] infections. Coronavirus entry into susceptible cells is a complex process which requires the concerted action of receptor binding and proteolytic processing of the S protein to promote virus−cell fusion [30]. Following the binding of SARS-CoV-2 to the host protein, the spike protein undergoes a two-step sequential protease cleavage eventuating the activation of S proteins [31, 32]. The S1 subunit binds to a cellular receptor while the S2 subunit mediates fusion of the viral and host membranes [33]. Activation of S protein for membrane fusion takes place through cleavage at the S1/S2 and the S2′ sites by host cell proteases such as furin, trypsin, cathepsins, transmembrane protease serine protease-2 (TMPRSS-2), TMPRSS-4, or human airway trypsin-like protease (HAT) [32, 34–36]. However, there are indistinct but functionally relevant differences between SARS-CoV and SARS-CoV-2 receptor binding due to which SARS-CoV-2 RBD has a significantly higher hACE2-binding affinity [37]. The high infectivity of SARS-CoV-2 is attributed to the ubiquitous expression of furin. Preactivated-furin aids the entry of SARS-CoV-2 in host cells even with low expression of TMPRSS-2 and lysosomal cathepsins [25].

Innate Response Activation

Upon gaining entry into the host cell through ACE2 and TMPRSS2 receptors, SARS-CoV-2 undergoes active replication during which pathogen-associated molecular patterns (PAMPs), such as single and double-stranded RNAs (ssRNA and dsRNA) are recognized by pattern recognition receptors (PRRs) like endosomal Toll-like receptors (TLR) 3 and 7, cytoplasmic receptors like retinoic acid-inducible gene I (RIG-I) like receptors (RLRs) and melanoma differentiation-associated protein 5 (MDA5) [4, 38]. PRRs present on immune cells such as dendritic cells (DCs) and macrophages lead to activation of type I interferon (IFN) genes [39–42].

TLR-3 senses dsRNA and complexes with the intracellular adapter proteins like TIR domain-containing adapter protein inducing IFN-beta (TRIF) and TNF receptor-associated factor 3 (TRAF3) to activate TANK-binding kinase 1 (TBK1) and inhibitor of NF-kappa B kinase epsilon (IKKε). Phosphorylation of IFN regulatory factor 3 (IRF3) by TBK1 and IKKε induces transcription of type I IFN genes [43]. TLR-7, on the other hand, senses ssRNA, via adaptor proteins myeloid differentiation primary-response protein 88 (MyD88) and TRAF3 to activate IKKα [44] resulting in

phosphorylation of IRF7. TLR-7-MyD88 complex stimulates NF-κB resulting in transcription of downstream proinflammatory cytokines and IFN gene expression [43].

RIG-I and MDA5 recognize short and long dsRNAs respectively [45, 46]. Both recruit and activate adaptor protein mitochondrial antiviral signaling protein (MAVS), which initiates the production of IFN signaling by TRAF3 and TRAF6 [47], thereby activating TBK1 complex [48, 49]. Phosphorylation and homodimerization of IRF3 and IRF7 by TBK1 complex, induces transcription of type I IFN genes. TRAF2/5/6-mediated activation of IKK complex activates NF-κB inducing transcription of proinflammatory cytokines [50, 51].

Increased expression of type I and III IFNs accompanied by other pro-inflammatory cytokines like tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), IL-6, and IL-18 constitute the first-line innate immune response and further activate adaptive immune response [25, 42, 52]. Type I IFN response is sufficient to control the spread and replication of virus at an early stage. But, the "smart pathogen" has evolved several immune evasion mechanisms to escape pattern recognition and downstream signaling [42, 53, 54].

Adaptive Immune Response

Various studies on SARS-CoV and other viral diseases have exhibited the clearance of pathogen by development of protective immunity [55]. This highlights the fact that optimum activation of CD4+, CD8+ T cells and neutralizing antibodies could possibly eliminate the infection and also produce long term immunological memory [56, 57].

As the first line of response gets activated, antigen presenting cells (APCs) like DCs, acquire the pathogen, carry out antigen processing and migrate to the draining lymph nodes (DLNs) [58]. In the DLNs, they are presented via major histocompatibility complex (MHC) to circulating naïve T cells [59–61] causing activation of virus-specific effector T cells. Several MHC polymorphisms are reported to be associated with disease susceptibility. For example, human leukocyte antigen (HLA) polymorphisms such as HLA-B*4601, HLA-B*0703, HLA-DR B1*1202 correlate to the susceptibility of SARS-CoV [62] whereas, HLA-DR0301, HLA-Cw1502 and HLA-A*0201 alleles are related to the protection from SARS infection [63]. Antigen presentation to T cells leads to production of antiviral cytokines (IFN-γ, TNF-α, IL-2), chemokines (CXCL-9, CXCL-10 and CXCL-11) and cytotoxic molecules (perforin and granzyme B) [64]. These inhibit viral replication, enhance antigen presentation [65], employ more immune cells at the site of infection and directly kill infected cells [66–69].

Both cell mediated and humoral immunity play a key role in clearance of infection. Humoral immunity functions through antibody production and complement activation [70, 71]. CD4+ T cells facilitate production of virus-specific antibodies via B cells. Detectable levels of IgM antibodies have been observed after 4 days of infection, peaking at day 20 and subsequently declining whereas, IgG antibodies have been detected after 7 days of infection, peaking at around day 25 and remaining for more than 30 days of infection [72]. CD8+ T cells trigger T cell-mediated cytotoxicity and T helper (Th) cells release proinflammatory cytokines. However, coronavirus can inhibit T cell functions by inducing their apoptosis [73].

The immune modulation of adaptive response by SARS-CoV-2 through manifestation of lymphocytopenia and dysfunctional surviving T cells triggers a cascade of hypercytokinemia also called as "cytokine storm" which reportedly causes an inflammatory injury to the lungs and subsequently respiratory insufficiency, leading to life-threatening acute respiratory distress syndrome (ARDS) and multiple organ failure [4]. The hyperinflammatory cytokine response comprises of excessive blood plasma levels of IL-2, IL-6, IL-7, IL-8, IL-10, granulocyte colony-stimulating factor (G-CSF), IP-10, Monocyte chemoattractant protein-1 (MCP1), macrophage inflammatory protein 1α (MIP1α), TNF and chemokines (CXCL8, CXCL9, CXCL10, CCL2, CCL3, CCL5) in SARS-CoV-2 infected patients [5, 74–79]. The dysregulated T cell makeup, especially increase in numbers of naïve T cells and decrease of memory and regulatory T cells is observed to be the prime reason of the resulting cytokine storm and as reported in several cases, may also be associated with relapse of infection [80, 81]. It is therefore, crucial to control the cytokine storm at early stages and restore the T cell balance. Interestingly, CD4+ T cells were seen to increase in recovering patients, indicating their prominent role in pathogen clearance [82–84]. All these findings prove to be a basis for the development of an effective multiepitope vaccine consisting of both B and T cell epitopes.

Immune Evasion Mechanisms of SARS-CoV-2

In addition to the immune pathways, SARS-CoV-2 has undefiable characteristic of evading immune system especially the innate immune response and dampening human defences. SARS-CoV and MERS-CoV putatively use several mechanisms to escape the pattern recognition and downstream signaling for better survival, which are presumed to be employed by SARS-CoV-2 owing to their comparable genomic sequence. As discussed previously, IFNs play a key role in controlling the infection. CoVs are known to employ multiple ways to evade the antiviral IFN responses. This includes inhibition of IFN induction, suppression of IFN and antiviral action of interferon stimulated gene (ISG)

products and avoidance of IFN response. Subsequently, it also downregulates MHC class I and class II molecules in infected macrophages or dendritic cells, resulting in impaired antigen presentation and diminished T cell activation [53]. Both SARS and MERS CoVs are known to express proteins that interfere with downstream signaling cascades [25]. The nucleocapsid protein of SARS-CoV is involved in immune evasion as it is seen to suppress RNAi in mammalian cells [85]. In the early stage of signaling cascade, N protein further antagonizes IFN induction [86]. SARS and MERS-CoVs circumvent the host detection of their dsRNA by replicating in the double membrane vesicles which are devoid of PRRs [87]. SARS-CoV ORF3b inhibits the production of type I IFN and ORF6 blocks the nuclear translocation of STAT1 [88, 89]. ORF4a, ORF4b, ORF5, and membrane proteins of MERS-CoV inhibit nuclear transport of IFN regulatory factor 3 (IRF3) and activation of IFN B promoter [90]. These viral proteins, except for ORF5, inhibit the expression of IFN-stimulated response element (ISRE) regulated genes. ORF4a downregulates the expression of NF-κB-stimulated genes whereas, ORF4b suppresses the interaction between IKKε and MAVS thereby, inhibiting the activation of IRF3 [91]. The membrane protein of SARS-CoV impedes the formation of IKKε complex which further suppresses the activation of IRF3 and 7, ultimately reducing the expression of type I IFN [90].

Non-structural proteins also play a role in immune evasion. For instance, nsp-mediated capping of the viral mRNA inhibits SARS-CoV detection by MDA5 and interferon induced protein with tetratricopeptide repeats 1 (IFIT1) [92]. Viruses elude adaptive immune responses by either conformational masking such as burying their RBDs in canyons [93] or recessed pockets [94]; or by glycan shielding, where components of spike proteins are hidden behind glycan clusters [95]. Therefore, understanding and overcoming the immune evading mechanisms is essential in developing treatment methods which are rapid, simple and efficient.

In Silico Studies for Identification of Probable Vaccine Candidates

Protection against SARS-CoV-2 is vastly associated with B cells producing persistent neutralizing antibodies and activation of virus-specific CD4+ and CD8+ T cells [96]. The strategy of an epitope-based vaccine is to include both B and T cell peptides owing to their role in antibody production, direct killing of infected cells and generation of long-term memory [55]. Owing to the rapid spread of COVID19 worldwide within a span of 6 months, experimental studies demonstrating T cell response are limited. Therefore, in silico analysis of SARS-CoV-2 proteome becomes all the more important to identify potential vaccine candidates. Several approaches are being used to identify potential regions in the SARS-CoV-2 proteome by scientists all over the world. Genomic similarities between SARS-CoV and SARS-CoV-2 have enabled identification of immunodominant regions in SARS-CoV-2. In addition, inclusion of experimentally validated conserved sequences between them, and de novo scanning of the entire proteome of SARS-CoV-2 comprises a full-proof approach to provide a more comprehensive list of potential vaccine candidates [55].

In silico approaches use bioinformatic tools which begin with mining the proteome for identification of antigenic peptides based on the sequence homology [97]. They further involve (Fig. 1): (a) prediction of strong-binding cytotoxic T cell lymphocyte (CTL), helper T cell lymphocyte (HTL), and B cell epitopes; (b) removal of self-peptides and antigenicity prediction; (c) population coverage analysis to take under consideration the polymorphisms of HLA; (d) epitope

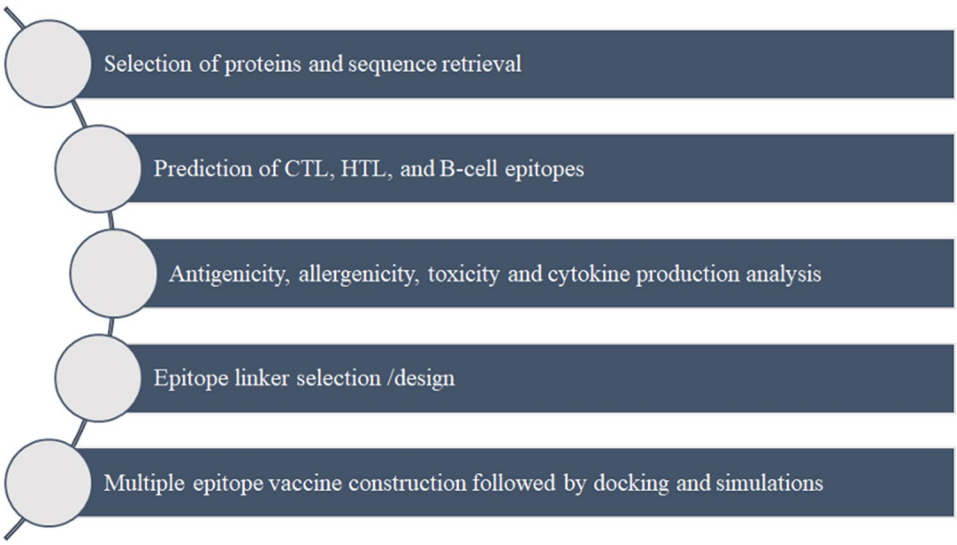

Fig. 1 Steps involved in a conventional in silico vaccine design approach

linker designing and multiple epitope vaccine construction followed by molecular docking and molecular dynamic simulation. Immunoinformatic tools mostly rely on the availability of *in vitro* assay data of MHC−peptide binding and cytokine assays. These methods have advantages over traditional vaccinology as they reduce both time and cost [98, 99].

Here, we have reviewed the different in silico vaccine design-based studies and gathered the immunogenic epitopes reported in such studies. We then looked out for the epitopes that were overlapping in different studies (Fig. 2). The accumulated epitopes were subjected to cytokine analysis to look for the epitopes having a preferential cytokine profile (Fig. 3). The epitopes with the preferred cytokine profile, that were also common in multiple independent studies, were shortlisted (Table 1).

Recently, Grifoni et al. identified regions in the COVID19 genome using two independent methods of homology and epitope prediction. B cell epitope prediction identified two regions from membrane protein (1–25 and 131–152) which also showed substantial IgM and IgG responses; and three regions from nucleoprotein (43–65, 154–175, and 356–404) [100]. 45 conserved T cell epitopes were also identified, most of which belonged to spike glycoprotein and nucleoprotein [100]. These epitopes can be further investigated for vaccine development.

In another study by Grifoni et al. peptide-mega-pools were created and tested in experimental studies [101]. This gives a glimpse of the T cell response of patients against

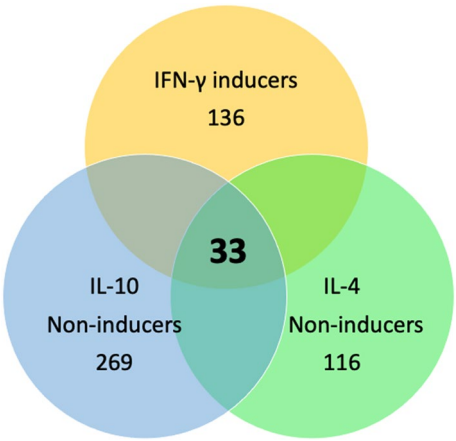

Fig. 2 Analysis of overlap of epitopes in all published studies to narrow down most potential candidates of SARS-CoV-2. The total of 443 epitopes retrieved from independent studies. After removing duplicates, 340 unique epitopes were found. Of these 70 were found common in at least 2 studies. Further down, 21 were common in at least 3 studies, 9 were common in at least 4 studies and 3 were common in at least 5 studies. Overlap of epitopes between independent studies using a variety of approaches illustrates their high potential of being most promising vaccine candidates

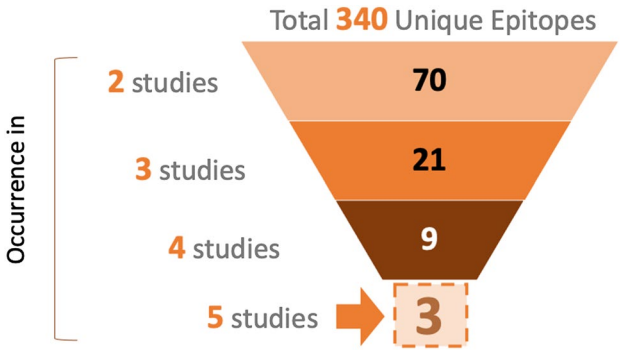

Fig. 3 Cytokine assessment studies of the 340 unique epitopes revealed 136 epitopes to be positive inducers of IFN-γ, 269 were non-inducers of IL-10 and 116 were noninducers of IL-4. 33 epitopes had all the three combinations i.e. IFN-γ inducer (Th1-inducer), IL-10 and IL-4 noninducers (Th2 noninducers). These 33 epitopes are predicted to be most favorable vaccine candidates based on cytokine predictions

SARS-CoV-2. Peripheral blood mononuclear cells (PBMCs) were taken from patients who had recently recovered from COVID19 infection, and stimulated with these peptide mega-pools synthesized for each of the 25 proteins of SARS-CoV-2. The CD4+ T cell response generated by membrane, nucleocapsid and spike were found to be co-dominant which is in contrast to other CoVs where only spike protein is responsible for maximum CD4+ T cell activation [101]. Significant CD4+ T cell response was also observed against nsp3, nsp4, ORF3s, ORF7a, nsp12, and ORF8. Although activation of CD8+ T cell response was observed by spike and membrane proteins, a more dominant CD8 response was generated by nsp6, ORF3a, and nucleocapsid proteins [101]. It can be inferred that spike as a vaccine candidate would be able to generate only adequate CD4 response suggesting that a cocktail of spike along with membrane, nsp6 and ORF3a is more likely to ensure optimum CD4 as well as CD8 response. The study also showed positive CD4 response in 40–60% of unexposed population reflecting some level of cross reactivity which still requires further validation [101]. Although the study was conducted on a small sample of hospitalized patients, it still provides missing insights into the actual T cell response of patients and can be exploited to identify T cell specific epitopes by various in silico methods.

In a study performed by Ahmed et al. homology between SARS-CoV-2 and SARS-CoV was analyzed extensively to find conserved epitopes between the two [16]. 49 B cell epitopes, most belonging to spike and nucleocapsid proteins were identified [16]. In order to efficiently narrow down the search for vaccine candidates for a high global population coverage, only those T cell epitopes were selected that were experimentally determined by positive T cell assays. 87 T cell epitopes were identified by this approach [16].

Table 1 List of 33 epitopes that are positive inducers of IFN-γ and noninducers of IL-10 and IL-4

Occurrence of epitopes in number of studies (Total 33 epitopes)	Peptide sequence	References
26 Found in only 1 study	NLDSKVGGNYNYLYRLFR	[100]
	QSIIAYTMSLGAENSVAY	[102]
	DSLSSTASALGKLQDVV	[100]
	GDAALALLLL	[100]
	ILLLDQALV	[100]
	SLPGVFCGV	[100]
	TLMNVLTLV	[100]
	FLAFVVFLL	[127]
	VLLFLAFVV	[127]
	VTLAILTALRLCAYC	[128]
	VVVLSFELLHAPATV	[106]
	IGMEVTPSGTWLTYH	[102]
	WNPDDY	[102]
	TWLTYHGAIKLDDKDPQF	[102]
	DEVNQI	[102]
	LLLTILTSL	[102]
	VNVLAWLYAAVI	[129]
	YLNTLTLAV	[102]
	NPAWRKAVF	[120]
	GETALALLLL	[130]
	ISNSWLMWLIINLVQ	[114]
	LTENLLLYIDINGNL	[114]
	WADNNCYLATALLTL	[114]
	MPYFFTLLL	[131]
	CLGSLIYSTAALGVL	[114]
	NQHEVLLAPLLSAGI	[114]
5 Found in exactly 2 studies	SVLLFLAFV	[102, 105]
	RRPQGLPNNTASWFT	[16, 102]
	YTNSFTRGV	[113, 132]
	GIYQTSNFR	[113, 133]
	YQTQTNSPR	[113, 115]
2 Found in exactly 3 studies	LALLLLDRL	[100, 134, 135]
	WTAGAAAYY	[16, 100, 102]

26 of these are found only in 1 study, 5 are found common in 2 studies and 2 are found common in 3 studies

Based on the epitope homology with SARS-CoV, Lee et al. identified epitopes which showed similarities with immunogenic peptides in the Immune Epitope Database (IEDB). These peptides also exhibited evidence of positive T cell assays and broad population coverage [102]. Wang et al. in their study, suggested the cross reactivity between experimentally confirmed immunodominant epitopes of SARS-CoV and SARS-CoV-2. 20 potential T cell epitopes were identified by them [103]. Similarly, sequence homology studies were also utilized by Kumar et al. to predict potential T cell candidates [104].

Abdelmageed and group used immunoinformatics approach for construction of a T cell-based vaccine. Out of all four structural proteins, envelope protein was found to be most antigenic [105]. 10 MHC class I and II restricted peptides with a significant global population coverage of 88.5% and 99.99% respectively, were identified as promising candidates [105]. Peele et al. constructed an in silico vaccine containing overlapping B and T cell epitopes from spike glycoprotein. Probable T cell epitopes were selected by studying antigenicity, allergenicity, toxicity, molecular docking and stability predictions. 18 T cell epitopes were finally included in the vaccine construct and their CD4+ and CD8+ T cell responses were confirmed by in silico immune simulations [106]. A vaccine construct containing highly antigenic HTL, CTL and B cell epitopes from nucleocapsid, membrane and spike proteins was modeled by Kalita et al. [107]. In a similar development of a vaccine design by Ojha et al. 6 B cell epitopes, 12 HTLs and 18 CTLs were selected using various computational tools. In another vaccine construct by Ahmad et al. shared B and T cell epitopes were selected. 2 epitopes from nsp8, 2 from 3C-like proteinase, and 1 from spike glycoprotein were considered to be the most promising candidates [108]. A similar multiepitope vaccine construct was also developed by Ismail et al. [109]. This kind of in silico multiepitope vaccine design can prove to be highly useful in the present times when experimental evidences are lacking.

Surface glycoproteins were studied by Baruah and Bose where 5 T cell and 8 B cell epitopes were obtained by in silico analysis. Interaction of CTL epitopes with MHC class I was seen to indicate their strong immunogenic nature [110]. A T cell epitope from spike protein showing maximum population coverage was proposed as a potential vaccine candidate by Joshi et al. [111]. Vashi et al. predicted 24 conserved immunogenic regions on the spike protein, 20 of which were found to be exposed on surface and could be promiscuous vaccine candidates [112]. Bhattacharya et al. identified 34 B and 38 T cell epitopes from spike protein using immunoinformatics approaches [113]. In silico epitope predictions have also been conducted by several other groups which are not yet peer-reviewed [114–119].

Understanding the population demographics, socioeconomic status, general immunity of the population and polymorphisms of HLA alleles are important issues to be addressed in developing an effective vaccine to vanquishing the global challenge of COVID19. In studies by Kiyotani et al. and Feng et al. epitope prediction was conducted specifically for Japanese and Chinese populations respectively [115, 120]. Apart from the conventional in silico vaccine design strategy, a recent study by Yang et al. has also proposed an artificial intelligence(AI)-based vaccine discovery framework. The group utilized it to design a multiepitope vaccine containing 16 B cell epitopes, 82 CTL epitopes and 89 HTL epitope from the spike protein of SARS-CoV-2 [121]. Similarly, the subsets of epitope hotspots were identified by another AI-based strategy that could be utilized in vaccine formulation [122]. Although scientists worldwide are looking for vaccine candidates with broad population coverage, a population-specific strategy could also be exploited. In the absence of tangible data of the actual immune response, computational approaches despite their limitations, continue to be our best shot to accelerate vaccine candidature studies [123].

As discussed earlier, in COVID19 patients, depletion of T cells and cytokine storm shift the equilibrium towards a disbalanced adaptive immune response. To restore the balance, optimal levels of CD4+, CD8+ T cells along with protective Th1 cytokines must be released for clearance of pathogen [55]. In our literature survey, we found a total of 443 immunogenic T cell epitopes. Out of 443, 340 were unique epitopes. Of these, 70 occurred in at least 2 independent studies, 21 occurred in at least 3 studies, 9 occurred in at least 4 studies and 3 occurred in at least 5 independent studies (Fig. 1, Supplementary Table S1). All 340 epitopes were passed through online servers—IFN epitope, IL4pred, and IL-10pred to assess their potential to induce IFN-γ, IL-4, and IL-10, respectively [124–126] (Supplementary table S1). Out of the 340, 136 were positive inducers of IFN-γ (protective Th1 response) and 204 were negative inducers (Fig. 2). 224 epitopes were seen to induce IL-4 (Th2 type) and 116 were noninducers (Fig. 2). 71 epitopes were found to induce IL-10 whereas 269 were noninducers of IL-10 (Fig. 2). Total 33 epitopes were found to be positive inducers of IFN-γ and negative inducers of IL-4 and IL-10 (Table 1, Fig. 3). Further, it was found that 7 of these epitopes overlapped in multiple independent studies making them the top most potential vaccine candidates (Table 1).

As discussed earlier, there have been multiple SARS-CoV-2 strains circulating globally and a vaccine should ideally provide protection across all the variants. The epitopes shortlisted in this reviewed study after cytokine analysis were found to be conserved across the different recognized variants of concern (alpha, beta, gamma, delta and omicron). This conservancy of epitopes relieves the pressure of vaccines getting ineffective with new emerging strains. Moreover, an epitope-based vaccine consisting of immunogenic portions from multiple proteins is expected to provide a broad spectrum protection.

Globally, several studies have been carried using in silico approaches, this review shortlists most potential candidates for development of COVID19 vaccine.

Conclusion

Occurrence of novel coronavirus SARS-CoV-2 has alarmingly raised a global public health and socio-economic emergency. Its catastrophic nature has set even the most developed economies with the best health care facilities rolling into a downward spiral. According to the International Monetary Fund, the global economy will take a plunge by 1–3% in the coming year due to COVID19. This unprecedented situation has set the world's scientific fraternity to race against time in order to contain the disease and find a cure. Advancements in technology, international collaborations and previous knowledge of SARS and MERS have remarkably expanded our understanding of the immune-pathogenesis of the virus within a short span of time. However, the biological complexity of the virus, various mechanisms of immune evasion and rapid mutation are major challenges that need to be addressed. Further, the high infectivity and long incubation period along with the burden of unidentified asymptomatic carriers also hamper disease containment. Therefore, it is imperative to elucidate the pathophysiology of SARS-CoV-2 for development of therapeutic strategies. Vaccine development strategies are directed with an aim to generate robust neutralizing antibodies coupled with a balanced cell-mediated response along with the desired milieu of cytokines. Therefore, developing a multiepitope-based vaccine seems a promising solution. Various groups of scientists have recently identified several epitopes which are capable of stimulating optimal levels of B cells, CD4+ and CD8+ T cells that can confer immunity

and long term memory. This review gives an account of the available literature of potential vaccine candidates obtained by in silico approaches. The study encompasses identification of antigenic T cell epitopes and the kind of immune response generated by them through their cytokine release. These particular epitopes in conjunction with an evidence of immunoprotective Th1 response can be treated as the most favorable candidates to be utilized in challenge studies and trials. We further propose them to be the strongest candidates of second generation of vaccines against COVID19 as they are derived from various different proteins as opposed to the first generation vaccines currently under trials which primarily focus only on spike protein.

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Declarations

Conflict of interest The authors declare no competing interests.

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