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Immunology and Vaccine Development against Covid-19

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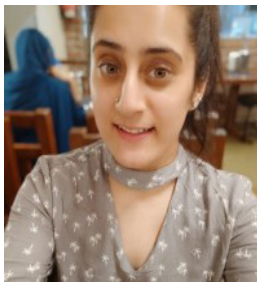

Author Biography

Mehak Vohra

Biography:

After Graduating in Biotechnology and Masters in Stem Cell Biology from Panjab University, I received my doctorate degree in Immunology and Stem Cell Biology in 2020 from Post Graduate Institute of Medical Education and Research, Chandigarh, India. My Ph.D. work was focused on evaluating the effect of umbilical cord derived mesenchymal stem cells for treatment of rheumatoid arthritis. During my Ph.D. I presented my work at National and International Conference and in 2018, at a conference, Immunocon, I was awarded the "Young Scientist Award" for my oral presentation.

In 2021, I joined Institute of Genomics and Integrative Biology, New Delhi, India, as Senior Project Associate. My main research interest lies in understanding host-pathogen interactions and providing new opportunities for therapeutic interventions. Thus, I look forward to continue to work in the areas which can have bench to bedside translation and have an impact on patient lives.

I am currently involved in studying the role of lipid droplet associated proteins, particularly perilipin 2 in functions beyond lipid metabolism in M. Tuberculosis infected macrophages. When not doing experiments, I enjoy my yoga classes, playing badminton, travelling and spending time with my loved ones.

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Abstract

The coronavirus disease 2019 (COVID-2019) pandemic caused by novel severe acute respiratory syndrome corona virus-2 (SARS-CoV2) has pushed the scientific community to face a challenge to find solution to its spread, specific therapeutic agents and an efficacious vaccine. In the past also, other similar viruses like SARS-CoV and middle-east respiratory syndrome (MERS)-CoV have made the world face pandemic like situations. These three coronaviruses causing pandemic like situation belong to beta-CoV and despite the similarities between their structure and genome, they differ significantly epidemiologically. These viruses were first discovered in 1960s and were named after their crown like shape appearance. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) was initially identified in Wuhan, China in December 2019 and since then it has spread globally and affected millions of people till date. Interaction between the viral spike protein and angiotensin converting enzyme 2 (ACE2) cell receptor causes the entry of the virus into the host cell. The virus shows its effect in form of flu like symptoms, pneumonia and respiratory tract infection. This review summarizes the epidemiology, pathogenesis along with the host immune system response to the virus and some of the vaccine candidates

INTRODUCTION TO CORONAVIRUS AND GENOMIC FEATURES:

Viruses are infectious particles containing RNA or DNA as a genetic material, which maybe single or double stranded. These viral particles cannot reproduce on their own and thus require a host cellular machinery to replicate and enhance the viral load (1). Corona Viruses (CoV) belong to the largest group of viruses that can infect mammals and other species. The coronavirus virion, which is a complete functional virus has a lipid envelope studded with club shaped fringe projections creating an image resembling solar corona. The single stranded positive sense RNA genome in CoVs is composed of six to ten open reading

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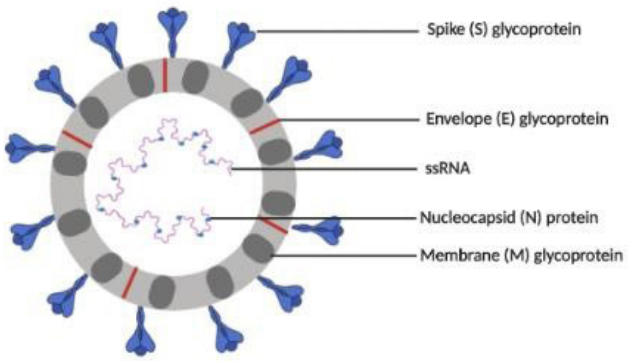
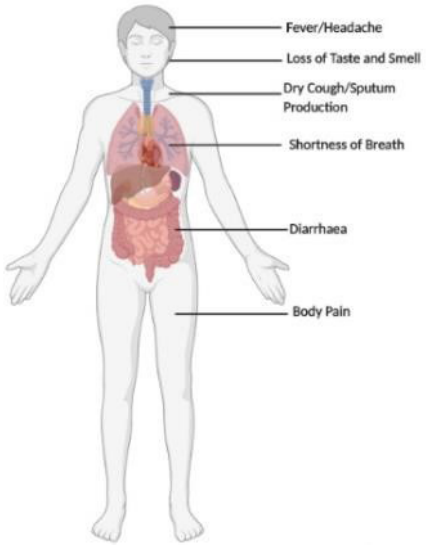
frames that encode the replicase protein, structural protein like spike membrane protein, envelope protein and nucleocapside protein. The spike protein helps in viral entry while membrane and envelope play critical role in viral assembly. In addition, some CoVs also contain gene encoding for hemagglutinin esterase. This enzyme possesses an acetyl-esterase activity that disrupts the sialic acid receptors present on the host cell surface and helps in attachment and invasion which increases the infectious properties of the virion (2) (Figure 1). In 1965, the first human CoV was isolated from people suffering from common cold. In the beginning of the 21st century, two new pathogenic CoVs were identified named severe acute respiratory distress syndrome (SARS-COV) and Middle East respiratory syndrome coronavirus (MERS-CoV). The world health organization declared it as first human to human transmissible pandemic disease in 21st Century (3) with morbidity and mortality at rates of 10% and 34% respectively . Almost after a decade, in December 2019, Wuhan, China reported several cases of pneumonia of unknown cause. It was identified as a novel beta coronavirus and was first called 2019-novel coronavirus (2019-nCoV). It was later renamed by the WHO as SARS-CoV2, because of its close resemblance with 2002 SARS-CoV.

The disease caused by this virus is termed as coronavirus disease 2019 (COVID-19). Due to the continuous spread of this disease worldwide, WHO officially announced the COVID-19 outbreak as a pandemic on March 11, 2020. The disease poses a great challenge to the whole world, as there is no specific treatment available.



Source and Mode of Transmission:

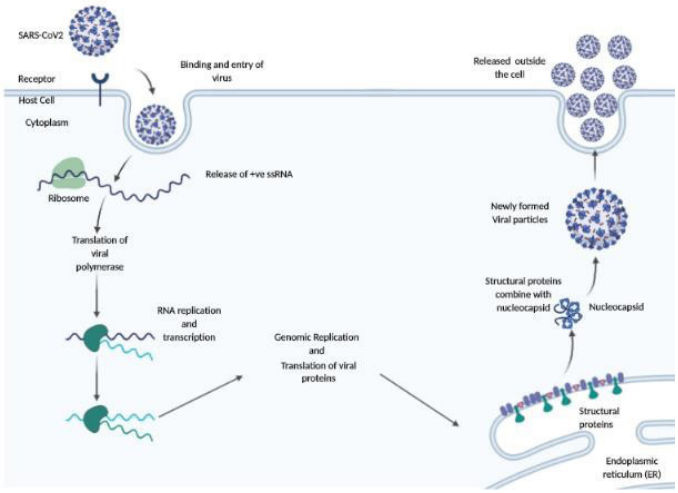
In Wuhan, China the beginning of the SARS-CoV2 pandemic began at the animal wholesale market. It is believed to have originated in animals and later jumped from animals to humans. Recent studies suggest bats are the main reservoir of this novel virus causing epidemic spread and presence of wild animals in seafood market supports this finding (4). Some other studies report human to human transmission as secondary cases began to be reported, which had no history of previous visit to the Wuhan animal market but had come in contact with the infected humans. Further, it has been shown that people infected with virus can spread to other humans much before any symptoms develop. In addition, asymptomatic individuals could spread the disease as well (5). On 13th January 2020, first non-chinese case of infection was reported from Thailand. The Chinese tourist had travelled to Thailand and had no history of visit to the market (3). Later cases from other countries like USA and France began to be reported, thereby confirming the spread through human to human transmission (4). Close contact between humans causes its transmission. Sneezing or coughing by an infected person has been well documented for the spread of the virus (6) through infectious droplets, which can settle in the mouth or nasal mucosa and lungs of another person via inhaled air. Currently, it is not very clear whether it can also spread via touching infected surfaces and subsequently touching mouth, nose and eyes, however, few studies have shown that SARS-COV2 remains viable in aerosols for minimum 3 hours and 24-72 hours on plastic or stainless steel surfaces (7). Virus can infect people of any age and symptoms differ from one person to another. The most common symptoms being fever, dry cough, fatigue, shortness of breath while other symptoms are sputum production, headache, diarrhea, lymphopenia and haemoptysis (7). Some individuals reported with reduced ability to smell (hyposmia) and taste (hypogeusia). The risk of death depends on age, severity of disease and underlying co-morbidities like chronic kidney disease, chronic lung disease, diabetes, cardiovascular disease, malignancy and hypertension (Figure 2). These symptoms usually appear 2 days to 2 weeks after being exposed to virus. It has been seen that many individuals experience mild symptoms or are asymptomatic during the COVID-19 pandemic (8). Thus, strategies need to be developed to minimize/inhibit the transmission of disease along with vaccine development.



Coronavirus: Infection and Replication

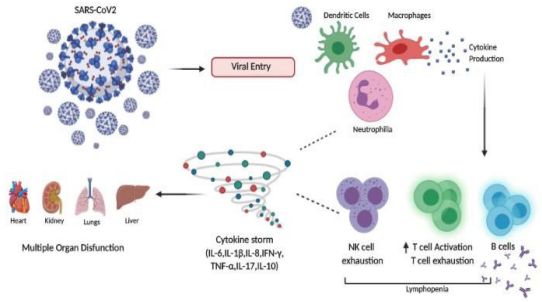
The spike (S) protein present on the cell surface of the virus helps initiate the entry of the coronavirus inside the target cell and is the main target of neutralizing antibodies upon infection. Studies in the past have shown that the function of the transmembrane spike glycoprotein depends upon its cleavage by host proteolysis enzymes into S1 and S2 subunits. The S1 subunit facilitates the viral attachment to the receptor, angiotensin converting enzyme 2 (ACE2), whereas S2 subunit governs the fusogenic activity of the virus-cell membrane. Additionally, the receptor binding domain (RBD) is present on the S1 subunit (10). In a few coronaviruses, RBD is present at the N-terminus region of the S1 whereas in SARS-CoV-1, it is situated at the Cterminus region (11). The striking structural similarity between the RBDs of SARS-CoV and

SARS-CoV2, it was speculated that SARS-CoV-2 might also use ACE2 as the receptor. In a seminal study by Shang J et al., describing the cell entry mechanisms of SARS-Cov-2, it has been shown that the SARS-CoV-2 spike binds to its receptor human ACE2 (hACE2) through its receptor-binding domain (RBD) and is proteolytically activated by human proteases. Using biochemical and pseudo virus entry assays, the investigators showed that the SARS-CoV-2 RBD supports the efficient entry into the host cell because it is preactivated by proprotein convertase furin, reducing its dependence on target cell proteases for entry. Although the SARS-CoV-2 RBD is more potent, yet it is less exposed than SARS-CoV RBD, because the hACE2 binding affinity of the entire SARS-CoV-2 spike is comparable to or lower than that of SARS-CoV spike. The high hACE2 binding affinity of the RBD, furin pre-activation of the spike, and hidden RBD in the spike potentially allow SARS-CoV-2 to maintain efficient cell entry while evading immune surveillance. These features may contribute to the wide spread of the virus. With the fusion of the membrane, virus gets entry into the host cell and releases its positive single stranded RNA into the cytoplasmic compartment, where the translation of ORF-1a and ORF-1b begins resulting in the formation of two large polyprotein-pp1a and pp1b. Polyproteins are then cleaved into 16 non-structural proteins by the activity of three functional proteases (12). This ultimately leads to formation of viral polymerase and other accessory proteins, which get incorporated into the golgi membrane or rough endoplasmic reticulum. Subsequently, nucleocapsid are formed by combining the +ve ss RNA with capsid protein (13). Lastly, shedding of the virus occurs which ultimately effects neighboring cells and are also released into the environment in form of respiratory droplets which potentially helps in spreading of the disease (Figure 3).



Receptor: Angiotensin-Converting Enzyme 2 (ACE 2)

Receptor recognition is the first step towards a viral infection to a host cell and its pathogenesis. Angiotensin converting enzyme 2 serves as a receptor for SARS-CoV. These are mainly expressed in human heart, kidney, lung parenchyma, intestinal epithelium and human endothelium, but is not expressed on T or B cells or macrophages in spleen or lymphoid. Angiotensin converting enzyme (ACE) plays a vital role in controlling the cardio-renal function and blood pressure, as it catalyzes the formation of angiotensin II from angiotensin I (14). ACE2 serves as a receptor for SARS-CoV2 and SARS-CoV whereas MERS-CoV binds specifically to Dipeptidyl peptidase 4 receptor 4 (DPP4) (15). In case of novel coronavirus, SARS-CoV2, functional evaluation was carried out to find out the potential receptors and it revealed that entry of the viral particle is increased in human cells expressing ACE2 receptor instead of DPP4. In addition, SARS-CoV2 binds to ACE2 with 10-20 folds higher binding affinity as compared to previously known SARS-CoV (16). Thereby suggesting easier transmissibility and increase susceptibility of SARS-CoV2 into the host cells. A study conducted by Finucane *et al* reveals that increased level of plasma glucose and a state of insulin resistance increases the expression of ACE2 in lung epithelial cells and act as a risk factor for morbidity and mortality in SARS-CoV2 infected patients (17). Another study by Holshue *et al* reported that stool samples from COVID19 infected patients when tested were positive for SARS-CoV2 thereby suggesting that the virus could also affect the gastrointestinal tract (18). While some latest reports have revealed, SARSCoV2 spread into the host cell depends on slicing of the trimeric S-protein by serum proteases known as Transmembrane proteases serine type 2 (TMRSS2). The TMRSS2 are highly expressed in human epithelial tissue lining the upper airway, bronchi and lower airways. Under normal physiological conditions, TMRSS2, helps in proteolytic cleavage of the epithelial sodium channel and regulation of sodium currents, while its exact function in lung epithelial cells is still not clear (19). Thus, further studies on viral particle along with its potential receptors to which the virus binds and its associated proteases will help us design anti-viral drugs and neutralizing antibodies which can help control the transmission of SARS-CoV2.



Immune Response to SARS-CoV2:

Impaired Type I IFN response:

Upon infection with SARS-CoV2, the host cell recognizes the whole virus or its surface epitopes and activates well-coordinated and rapid innate and adaptive immune response, which represents the first line of defense against viral infection. Innate immune response mainly consists of natural killer cells, macrophages, DCs and molecules such as type I interferon, chemokines and cytokines. The key role of innate response is to combat the virus by inhibiting its replication via type I interferon response. Type I IFN production is induced by the pathogen recognition patterns (PRRs) like Toll-like receptors (TLRs) - 3, 7 and 8, which are present on immune cells.

Toll/IL-1 receptor mediates the TLR signaling cascade and it contains adaptor molecules like MyD88 (myeloid differentiation primary response gene 88), TIRAP (TIR- domain containing adaptor protein) or TRIF (TIR-domain containing adaptor). The TIR complex plays a critical role in inflammatory immune responses via production of pro-inflammatory cytokines, type I IFN and up-regulation of co-stimulatory molecules. In case of SARS-CoV and MERS patients, it was observed that they had impaired IFN response, as no type-I IFN could be detected in the lungs and serum of these patients (20,21). A similar pattern of impaired antiviral type-I IFN response was seen in COVID-19 patients. This was revealed recently, wherein the whole blood transcriptome and cytokine storm profiling was done and it indicated that type I IFN activity was low in severe COVID-19 patients and expression of interferon stimulated genes was downregulated (22). Though the exact mechanism of impaired Type I IFN response is not fully understood in any of the three diseases (SARS-CoV, SARS-CoV2, MERS-CoV) so far, yet few studies suggest viral components itself helping the virus escape the antiviral response. In case of

SARS-CoV, its nucleocapsid protein has direct inhibitory effect on IFN induction and its ORF3b also indirectly affects the phosphorylation of IRF3, thus affecting the production of IFN (23). While in MERS-CoV, the ORF4, ORF5 and membrane protein have effect on the nuclear trafficking of IRF3 and activation of the IFNB promoter (24). In addition, comorbidities such as obesity can also inhibit the type-I IFN production via leptin and SOC3. Channappanavanar *et al* has shown in a mice model infected with SARS-CoV, that viral replication is accompanied by delayed type I interferon (IFN-I) signaling that orchestrates inflammatory responses and lung immunopathology and death. Type I IFNs remained detectable until after the peak of viral titers, and delayed IFN-I signaling promoted the recruitment of pathogenic inflammatory monocytes/macrophages, resulting in elevated lung cytokine and chemokine levels, vascular leakage and impaired virus-specific T cell responses (25). Currently, thirteen clinical trials are ongoing to evaluate the efficacy of IFN Type I treatment in patients with COVID-19 infection

(www.clinicaltrials.gov).

Impaired NK Cell Cytotoxicity:

The natural killer (NK) cells upon activation by various stimuli including IFN-I, show antiviral properties by either directly killing the target infected cells or indirectly via activating the macrophages. Till date, no clear-cut data about role of NK cells in clearing the coronavirus has been fully elucidated. However, few studies have shown lower levels of NK cells in blood of severe SARS-CoV and SARS-CoV2 patients as compared to normal. These findings do not clarify, whether this lower count is due to redistribution of the NK cells at the infected sites or cell death. Zheng *et al* have shown in his work that the expression of NK group 2 member A (NKG2A) receptor is increased in COVID-19 patients as compared to healthy controls. The NKG2A is a receptor that transduces inhibitory signals and suppresses T cell and NK cell cytokine secretion and cytotoxic function (26). It was also observed that the percentage of T and NK cells expressing the activation markers CD107a, IFN-γ, IL-2 and TNF-α was significantly reduced. Thus, the data from this study suggests that patients with severe COVID-19 are characterized by functional exhaustion of the peripheral NK cells and CD8+ T-cells. A similar pattern of NK cell effector function loss is observed in a condition called hemophagocytic lymphohistiocytosis (HLH). In HLH, elevated levels of IL-6 and IL-10 are thought to impair NK cell cytotoxic function, while IL-12 and IL-18 cause NK cell exhaustion or induce cell death (27). These data suggest that impairment of NK cells function in COVID-19 patients might be attributed to hypercytokinemia. By contrast, when bronchoalveolar lavage fluid (BALF) samples from COVID-19 severe and mild patients was taken for scRNA seq analysis, it revealed higher amount of NK-cells as compared to control, suggesting NK cell trafficking into the lungs (28,29). Taken together this data suggests that upon SARS-CoV2 infection, NK cells exit the peripheral blood and move to the lungs where they potentially contribute to local inflammation and injury and the NK cells that remain in the circulation display an exhausted phenotype that facilitate the virus spread to the other organs.

Activation of Macrophages and Dendritic Cells:

Macrophages are a vital component of inflammatory reaction and the balance of their activation plays an important role in determining their fate of infection. They are classified as classically activated M1 type that produce pro-inflammatory cytokines (IL-6, IL-1β and TNF-α) and alternatively activated M2 type which secrete anti-inflammatory cytokines (IL-10 and TGF-β). In case of COVID-19 patients, macrophages are thought to be major players in the production of inflammatory cytokines like IL-6, TNF-α and IL-1β. Studies conducted on BAL fluid from COVID-19 patients revealed that more than 80% of total cells are monocyte-derived macrophages (MDM) rather than alveolar macrophages and produce large amount of cytokines involved in inflammatory cytokine storm typical of the disease. Interestingly, in a mouse model of SARS-CoV infection, removal of inflammatory macrophages protected the animals from the lethal infection without affecting the viral load (25). A few of pathways that trigger the hyperactivation of macrophages in SARS-CoV have been shown and considering the similarities between SARS-CoV and SARS-CoV2, it might have similar underlying mechanisms in COVID19 patients. Usually macrophages recognize viruses with RNA genome through PRR, which on engagement activate downstream pathways like NF-κB and IRF 3/7, which ultimately lead to production of pro-inflammatory cytokines (30). SARS-CoV ORF8b and its components like viroprotein A, E protein, ORF3 activate the nucleotide binding domain like receptor protein 3 inflammasome (NLPR3) in macrophages (31,32). Secondly, dysfunctional NK cell and cytotoxic T lymphocyte (CTL) response might also result in hyperactivation of macrophages with impaired viral clearance and excessive IFN-ϒ production (33). In addition, IFN-ϒ production from dysfunctional NK cells and CTLs bind to the IFN gamma receptor (IFNGR) present on the surface of macrophages and cause phosphorylation of signal transducer and activator of transcription 1 (STAT1), which promotes the increase in expression of IFN-stimulated genes and pro-inflammatory cytokine. Thus, elucidating more pathways will help develop potential therapeutics for COVID-19 disease.

Apart from macrophages, dendritic cells also play a key role during viral infection, wherein some SARS-CoV peptides are presented via MHC-II to CD4+ T-cell. An association between, HLA haplotype and coronavirus infection has been reported in SARS-CoV and MERS-CoV cases while no such pattern has been reported in SARS-CoV2 infection as yet. In a recent report, the blood plasma from COVID-19 patient was able to block the expression of HLA-DR on CD14+ monocytes which was restored effectively on inhibiting IL-6, thereby suggesting that decreased HLA-DR expression in SARS-CoV2 patients is due to hyper inflammatory condition (34). Additionally MHC expression is found to be reduced during the infection due to epigenetic modifications of downstream molecules (35). A similar pattern of decreased MHC expression due to epigenetic modification in the calnexin promoter is seen in cancer cells, which helps it to evade the immune response (36). So far, we understand that a decrease in the innate antiviral response along with hyper-inflammation could be one of the causes of COVID-19 severity.

T cell response against coronavirus:

The role of T cell responses to respiratory coronaviruses like MERS-CoV and SARS-CoV has been reviewed extensively, while for SARS-CoV2, wide array of evidences are being gathered. Marked lymphopenia is observed in SARS, MERS as well as SARS-CoV2 patients. Especially in COVID-19 patients requiring intensive care unit (ICU), the counts of total lymphocytes, CD4+ and CD8+ cells lower than 800, 300 and 400/µl respectively are observed (37). The exact mechanism for lymphopenia in severe COVID-19 patients is unknown, but a few possible mechanisms that have been delineated include, chemokine production mediated redistribution, bone marrow suppression, virus mediated destruction, sustained Type I IFN response and high level of glucocorticoids leading to T-cell apoptosis (38). A study, conducted on more than 1000 Chinese people, reported that low count of circulating lymphocytes is linked to the disease severity (39). While the BALF samples from fatal SARS and COVID-19 autopsies reported low lymphocytic infiltration into lungs, although mild COVID-19 were reported with abundant CD8+ T-cells. Thus, excluding the possibility of lymphocytopenia because of chemokine mediated redistribution, it also explains unrestrained viral replication in the lungs of severe COVID-19 patients due to lack of infiltrating CTLs. In case of MERS-CoV, it has been noticed that T-cells get invaded by the virus which leads to their apoptosis. Such evidence is lacking in case of SARS-CoV and SARS-CoV2 infections, although apoptosis of lymphocytes has been recently reported in the spleen, lymph nodes and lymphoid tissue of the gut in COVID-19 patients (35,40). Recent data, has suggested expansion of myeloid derived suppressor cells (MDSCs) in fatal SARS-CoV infection along with decreased NK and T cells, thereby suggesting that the impaired cytotoxic functions might be related to MDSCs (38).

In addition, CD8+ CTLs upon activation and differentiation migrate into the infected site and cause the release of perforin and granzyme to destroy the virus-infected cells. In case of COVID19, a decreased proliferation of Granzyme B+ and CD107a+ CD8+ cells have been reported, thereby pointing towards an impaired cytotoxic activity of CTLs. However, increased expression of activation markers: the sCD25 on CD4 T cells, increased percentage of CCR4+ CCR6+ Th17 cells, lower T regulatory (Treg) cells, has been reported thereby indicating T-cell hyperactivation (41). The possible reason for this would be constant antigen stimulation and presentation caused by NK cells and CTL exhaustion. Apart from effector T cells, role of memory T-cells has also been delineated in a case study of SARS recovered patients, wherein memory T-cells elicited a response against the S protein in 60% of the recovered individuals (42). In recent reports, from COVID-19 patients reduced memory and Treg cells have been seen, which might aggregate the inflammatory response leading to cytokine storm and enhance tissue damage (43). In a mouse model, CD4+ memory T-cells were used as a vaccine and its restimulation with SARS-S366 peptide produced IFN-Ɣ and recruited CD8+ T cells for rapid viral clearance and thereby impart protective response against human coronavirus (43).

Cytokine Storm:

Cytokine storm occurs when rapid and massive production of the various cytokines occur after infection or autoimmune disease, which can lead to multi-organ failure and death. Like in SARS and MERS presence of lymphopenia and cytokine storm may have a significant role in pathogenesis of COVID-19 also (44). In SARS, immune cells triggered the production of proinflammatory cytokines like IL-6, IFN-α/γ, TNF-α and in MERS-CoV though delayed but increased production of IL-6, IL-8, IL-1β, IFN-α was observed (45). In case of COVID 19 patients, 30 kinds of cytokines were observed in serum, with few signature cytokines consistent with SARS and MERS are IL-6, TNF-α, MIP-1α, GM-CSF, IL-4, IL-8. IFN-γ, IL-2 and chemokines like IP-10, CXCL5, CXCL1 and CCL2/MCP1 (46–48). Some cytokines like IL-6, IL-10, IP-10, MCP-3 are implicated to be linked with disease severity. Amongst all the cytokines, role of IL-6 has significantly evolved in COVID-19 patients as well as SARS and MERS cases (49). In case of COVID-19, increased IL-6 contributes to increased vascular permeability, downregulation of perforin and Granzyme production by NK cells and triggers the monocytes recruitment (50–52). This cytokine storm is responsible for commencing of viral sepsis followed by lung injury induced by inflammation, which is related to other complications like acute respiratory distress syndrome (ARDS), pneumonitis, sepsis shock and organ failure. Various therapeutic measures are being used to control the signaling cascade for cytokine production.

Humoral response to Coronavirus:

Humoral response against SARS-CoV2 involves the production of IgG and IgM antibodies, a pattern similar to other coronavirus infections. An early appearance of IgM and IgA by day 5 from the appearance of initial symptoms have been shown in 85% and 93% cases respectively, while IgG could be detected only by day 14 in 77% cases of SARS-CoV-2 (53). A similar pattern of IgA, IgG and IgM antibody detection at different time points in SARS-CoV infected patients has also been seen in earlier studies. The anti-S-RBD IgG could be detected in all of the cases, while anti-N IgG and anti-S-RBD IgM were detected only in about 90% of cases (54). A number of virus specific neutralizing monoclonal antibodies or fragments targeted to S protein in SARS-CoV and MERS-CoV have been developed, but none of them have been assessed in clinical trials (55). Till date no SARS-CoV2 neutralizing MAbs have

been available for human use. Hence, testing for neutralizing antibodies is therefore necessary to rule out cross reactive antibodies directed against S-proteins of SARS-CoV and SARS-CoV2.

Vaccine development:

SARS-CoV2 is a highly contagious virus and it's spread as on today can only be prevented via social distancing, frequent hand wash, disinfection and by universal wearing of mask in public. Despite preventive measures, these strategies leave the individuals without immunity against the virus and makes them susceptible to infection. Vaccination against COVID-19 seem to be imperative to stop the spread of pandemic worldwide. A vaccine produces an immunity in the individual which involves the recognition of the vaccine-containing agent as foreign to the body, causing its destruction and lead to the formation of immune memory. The immune memory developed, leads to faster and effective defense responses by the body in case of subsequent contact with the similar pathogen. The ideal vaccine is one that is effective in preventing or reducing the severity of the disease, provides long-term protection against the disease, requires minimum number of administrations, causes minimal or no side effects , has long shelf life and lastly can be produced on a large scale and be available to the populations at a subsidized rate. Efforts are being made globally to develop a vaccine and many candidates are already there in various stages of clinical trials. Usually there are three stages in vaccine development- exploratory, preclinical and clinical stage. In the exploratory stage, an appropriate antigen of the disease for which vaccine needs to be produced is identified or developed. While in pre-clinical stage, animal models are used to test the safety and immunogenicity of the candidate vaccine. The final is the clinical stage where trials in different phases are conducted. In Phase I, small number of healthy subjects are voluntarily administered with the vaccine to test the safety of the vaccine. Once the phase I is cleared, it moves to Phase II where a larger group of several hundred diseased or high-risk individuals are tested along with a placebo group. The aim of this phase is to assess the vaccine's immunogenicity, dose, route of administration and its schedule. This phase can take two or more years. After successful Phase II, in Phase III, the most promising vaccine candidate's safety and efficacy is tested in a very large group of people (3000-50,000). After Phase III comes the approval from the regulatory authorities before the vaccine reaches to the population after large scale production. In USA, Investigational New Drug of the Food and Drug Administration and the European Medicines Agency (EMA) in the European Union are some of the regulatory authorities. In the last and final phase IV, pharmacovigilance and monitoring long term adverse effects of vaccine is done. Usually for the development of a vaccine, on an average, it takes 10-15 years but in pandemic situation, where there is urgent requirement of the vaccine, the process of clinical trials is fast-tracked and there occurs overlapping of the clinical trial phases and the entire vaccine development time can be brought down to 12-18 months (56). As per WHO reports, 166 vaccine candidates are in pre-clinical stages, 56 are in clinical evaluation phase. Four vaccine candidate have cleared phase III trial-

Moderna's mRNA-1273, Pfizer/BioNTech's BNT162b2, Gamaleya's Sputnik V vaccine University-Oxford & AstraZeneca's AZD1222. Among most of the vaccines being developed, 6% are inactivated virus based, 13% RNA based, 8% DNA vaccine, 33% protein subunit, less than 2% live attenuated and 9% viral vector based vaccines (57). Traditionally vaccines manufactured were either inactivated, live attenuated or subunit but various institutions are now trying next generation techniques like RNA, DNA, Vector based RNA and messenger RNA bases vaccines. The nucleic acid vaccines being developed for SARS-CoV2 are mainly S protein based. Most of the vaccines are being developed in USA, China and Europe. In India, two candidate vaccines have been development in Bharat Biotech and Zydus Cadila biotech companies. Serum institute of India in collaboration with Oxford University and Astra Zeneca are also working on development of a vaccine.

Current Vaccines:

Sinovac

Sinovac Research and Development Co., Ltd. is leading an ongoing Phase III clinical trial (NCT04456595) on the vaccine PiCoVacc. The vaccine is based on an inactivated virus and contains 3ug/0.5ml of inactivated SARS-CoV2 virus along with aluminium hydroxide as adjuvant. In this Phase III trial, which is a double blinded placebo-controlled trial, two doses of intramuscular injection with a gap of 2 weeks between the injections, will be given. Patients will be monitored over the course of 12 months to measure the outcome of the trial. Patient recruitment in two age groups: Adults (18-59 years) and Old (60 years and above) has concluded in October 2020. In Phase I (NCT04352608, NCT04282574), seroconversion rate of neutralizing antibodies against novel CoV was tested using micro-neutralizing assay in serum and it was found that seroconversion rate as high as 90% was seen within 14 days of immunization.(58)

University of Oxford:

Jenner Institute of University of Oxford have developed a vaccine, ChAdOx1 (AZD1222). The vaccine contains sequence of SARS-CoV-2-S-Protein with a transgenic, non-replicating chimpanzee adenovirus-based vector. Previous studies have reported single dose of ChAdOx1

MERS, a chimpanzee adeno (ChAd)-vectored vaccine platform encoding the spike protein of MERS-CoV, protected nonhuman primates against MERS-CoV-induced disease. It has now completed its Phase I clinical trial in case of MERS-CoV cases (NCT04324606) (59). For SARS-CoV2, it has entered into Phase III clinical trial. It is a double blinded placebo-controlled trial which will enroll 30,000 participants. Two intramuscular doses will be given to adult's ≥18 years and old at a gap of 4 weeks between the injections. Each participant will receive a dose of 5*10^10 viral particles of AZD1222. In this phase, it plans to evaluate the safety, tolerability, reactogenicity, humoral and cellular immunity of the ChAdOx1 nCoV-19. The Phase I/II clinical trial of this vaccine was conducted in five trial sites in the UK. Healthy adults aged 18–55 years with no previous history of SARS-CoV-2 infection or of COVID-19-like symptoms were randomly assigned to receive a single dose of intramuscular injection of ChAdOx1 nCoV-19 at a dose of 5×10^{10} viral particles or Meningococcal conjugate vaccine (control). The ChAdOx1 nCoV-19 showed safe and well tolerated profile and

homologous boosting increased antibody responses. No serious adverse reactions to ChAdOx1 nCoV-19 occurred (NCT04324606). These results, together with the induction of both humoral and cellular immune responses, supported large-scale evaluation of this candidate vaccine in next phase of clinical trial.

Moderna Vaccine:

This is an mRNA-based vaccine, the candidate mRNA-1273 is a lipid nanoparticle (LNP)encapsulated mRNA that encodes full-length, prefusion stabilized spike (S) protein of SARSCoV-2. The vaccine is manufactured by ModernaTX, Inc, USA. The vaccine was initially tested in primate rhesus monkeys and revealed Type I helper T cell based CD4 T-cell response in animals, while low Th2 or CD8 cell response was found. After the testing in animals, the manufacturers along with National Institute of Health (NIH) and National Institute of Allergy and Infectious diseases (NIAID) conducted Phase I clinical trial (NCT04283461), in which healthy volunteers starting at age 18 years were given two doses of intramuscular injection on Day 1 and Day 29. All of the participants showed time and dose dependent antibody response to the full length S-2 protein. No adverse events occurred apart from fatigue, chills, headache and pain at the injection site (60). Following Phase I trial, Phase 2a (NCT04405076) randomized, placebo controlled, dose-confirmation study was conducted to evaluate the safety, reactogenicity, and immunogenicity of vaccine in adults aged 18 years and older. In this trial, 600 healthy volunteers were enrolled and injected with either 50µg or 100µg dose of the targeted vaccine and it was found that the response was better at a higher dose. Currently, this vaccine has entered into Phase 3 trial (NCT04470427), wherein 30,000 participants will be enrolled and they will receive an intramuscular (IM) injection of 100 microgram (ug) on Day 1 and on Day 29.

CanSino Biologics Inc, China:

An adenovirus type 5 vectored COVID-19 vaccine, the Ad5-nCoV is the vaccine developed by CanSino Biologics Inc, and Beijing Institute of Biotechnology, China, which is also about to enter clinical phase III trial. Phase I of the trial (NCT04568811) was a single-center, open-label phase I clinical trial of booster vaccination in healthy 18 to 60 years of age, inclusive, who has been prime vaccinated with adenovirus type-5 vectored COVID-19 vaccine. It revealed that higher dose of the vaccine was associated with severe adverse reactions within 14 days after booster vaccination. Subsequently, in Phase II (NCT04566770), in which 481 participants were enrolled and evaluated for the immunogenicity and safety of the vaccine at a lower and middle dose. In this the participants were injected intramuscularly with two doses $1*10^{11}$ viral particles and $5*10^{5}$ virus particles and rest were in placebo group. Participants in both the groups showed no adverse reactions and also either cellular or humoral immune responses at day 28 post vaccination. In Phase III trial (NCT04526990), it has started recruiting participants with an intent to enroll a total of 40,000 individuals in double-blind, placebo-controlled trial. Half of the participants will receive a single dose of the vaccine intramuscularly and will be evaluated for the efficacy, safety and immunogenicity of Ad5-nCoV in Adults 18 Years of Age and Older. The primary data from the study is estimated to be completed by Dec 30, 2021. (61)

Pfizer and BioNTech:

BioNTech and Pfizer initially developed 4 mRNA-based vaccines, out of these two vaccines BNT162b1 and BNT162b2 were found to be safe in the initial pre-clinical trials. BNT162b1, encodes for the SARS-CoV-2 receptor-binding domain (RBD) and to increase its

immunogenicity it is trimerized by the addition of a T4 fibritin foldon trimerization domain. While, BNT162b2, encodes for the SARSCoV-2 full-length spike, modified by 2 proline mutations (P2 S) to lock it in the prefusion conformation to increase its potential to elicit virusneutralizing antibodies. When evaluated in Phase I study, enrolling 18-55 years of healthy adults (NCT04471519), BNT162b1/2 induced a high, dose-dependent neutralizing antibody titer along with RBD binding IgG concentration after second dose, 21 days apart given intramuscularly. This was further accompanied by CD4+ and CD8+ T cell responses after administrating two doses of 1-50µg of BNT162b1/2. In addition, interferon-γ was produced by a large fraction of RBD-specific $CD8^{+}$ and $CD4^{+}$ T cells. This ability to elicit both humoral and cell-mediated antiviral mechanisms makes BNT162b1/2 a promising vaccine candidate. In its Phase 2/3 trial, they plan to enroll 30,000 participants between the age group of 18-85 years of age. Recently, Pfzier and Biotech have announced 95% efficacy of the vaccine candidate (BNT162b2), which is consistent amongst different age groups, gender, race and ethnicity. Though the data still has to be produced to different regulatory authorities, but it gives hope and confidence to the people regarding the vaccine development against SARS-CoV2.(62)

Limitations:

Several challenges are being faced while developing a vaccine for novel coronavirus SARSCoV2. The safety and efficacy of all the candidate vaccines are under question till the time the entire data from Phase III clinical trial is not examined properly. The testing of novel vaccines generally happen in animal models first but it has been observed that standard inbred mice are not susceptible to SARS-CoV2 infection as the expression of human and mice ACE2 receptor is different. Thus, transgenic mice are used in such setup either hACE2 transgenic mice or primate macaques model, but constant steady breeding and timely supply of these animals to the researchers is another challenge (63). In addition, finding the correct antigen dose, number of doses, booster requirement, interval between the boosters, testing it in children and pregnant females along with difference in ethnicity and geographical patterns are some of the challenges that need to be addressed. Even if we overcome these challenges, a mass production and making the vaccine available world-over and administrating it globally is another major challenge, which is going to be faced in coming times.

Conclusion:

The world has seen three outbreaks due to coronavirus. The epidemiology, transmission, pathogenesis and treatment regime still remain undiscovered. Raising awareness about this contagious virus, is the only way currently to prevent its spread. Each country has laid down its regulatory guidelines to break the chain of community spread. Currently, antiviral drugs, monoclonal humanized antibodies against IL-6, FDA approved molecules like Baricitnib, Ritonavir, Chloroquine/

Hydroxychloroquine etc. are being used to treat COVID-19 patients but with only limited success. Vaccine development along with general measures of social distancing and maintenance of personal hygiene can help prevent and stop the pandemic.

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