HOST IMMUNITY AND VACCINES

COVID-19

A WHITE PAPER

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FOREWORD

Viruses are older than the mankind and are more numerous than all other forms of life put together. Although their existence has been known to man for several centuries, the arrival of SARS-CoV-2 towards the closing days of 2019 as a new virus of exceptional virulence and pathogenicity led to a worldwide crises. Due to its high direct mortality for such a contagious disease, COVID-19 placed extreme pressures on the healthcare systems globally. Further, an incomplete information about the virus behaviour led to a wildly disproportionate reaction from both the general public and the health authorities. The World Health Organization (WHO) had to declare it a pandemic of exceptional significance.

The togetherness witnessed between the scientific community and the medical professionals with the single most objective of saving human lives by creating the minimum infrastructure needed for the purpose has been unprecedented and never witnessed before. Nations had to declare complete lockdowns of all commercial, economic and social activities and this created both direct as well as indirect pressures of highest proportions. Augmenting health care infrastructure, adequate availability of hospital beds, trained manpower including doctors, nurses and other healthcare workers became a global challenge.

For the Indian healthcare authorities, this was a gigantic task and the systems had to be activated on multiple fronts. The immediate challenge for us was to set up efficient diagnostic systems to detect and limit the spread of the virus. The Make-in-India *mantra* provided by our honourable Prime Minister became the guiding principle for the Government to incentivise public health measures.

The Indian Council of Medical Research (ICMR) played a crucial role in augmenting COVID-19 testing facility. From a mere one molecular testing laboratory in January 2020 at their National Institute of Virology in Pune, a whopping 2,240 laboratories were established by December 13, 2020. The ICMR validated over 1100 diagnostic commodities for COVID-19 at its 30 validation centres. A total of 552 test kits were approved, of which >70% were indigenous. Due to this and under the Atmanirbhar Bharat initiative, the cost of RT-PCR testing was drastically reduced from Rs 4200 to a more affordable figure of Rs 450.

Cond....

From conducting just ten tests per day on January 30, India reached a figure of over 12 million tests per day by mid-December last year. This is the highest number of tests performed by a single country surpassing even the United States' efforts.

Similar to the efforts on the laboratory diagnostics, our scientists, medical professionals, innovators and entrepreneurs in the industry, health planners and administrators acted in a highly coordinated manner to turn the pandemic challenge into an opportunity. The goal was how quickly to exit from the pandemic, keeping the morbidity and mortality to as low as possible.

The immediate task was three-fold. The first relates to the social sciences front to spread information about the disease among the masses at large to make them understand the behavioural and social implications of the pandemic. The challenge was to enforce a countrywide lockdown and make people realize the importance of observing the COVID appropriate behaviour including social distancing, mask wearing while outside and frequent hand washing. The call made by the honourable Prime Minister for a *Junta curfew* on March 22, followed immediately by the first lock down beginning on March 25 2020 was duly acknowledged by the countrymen in letter and spirit. This was a highly satisfying experience, much appreciated internationally.

The second most important challenge for the policy planners was to prepare a model for providing hospital admissions and efficient treatment to those getting infected and thus save lives in the short term. This was a gigantic task considering the huge gap that existed in the demand and supply, and inadequacies in the health infrastructure. The country hardly manufactured her own ventilators, personal protection equipment (PPE kits), adequate oxygen supplies and other infrastructure needed for the care of the sick in hospitals. It is a matter of pride for us that today India stands fully self-reliant in all of these needs and is even in a position to export to other countries.

The third and by far the most important task was to encourage the scientific community and the medical professionals towards the need to create in-house vaccines against COVID-19 and manufacture the same in the country. It was clear to us all that vaccination was the only way to achieve herd immunity and save our population from the dreadful impact of the pandemic. Again it is a matter of pride for us in the Niti Aayog that the industry, entrepreneurs and the scientific community got together in the spirit of unmatched collaboration and already created two very effective vaccines, both manufactured in India. Further, there are at least four more vaccines against the coronavirus in various stages of testing and development which will together fulfil the goal of vaccinating everyone and achieve community immunity against the novel virus.

Truly, while the year 2020 has been a year of challenge, 2021 offers new hope and confidence. Hopefully the life will return to normal sooner than later. I feel so happy that the Indian National Science Academy (INSA) has decided to bring out a 'white paper' on the very important topic of COVID-19 immunity and vaccination. I congratulate the authors led by Prof. Narinder Mehra for their excellent efforts in this endeavour.

January 30, 2021

(Vinod Paul)

प्रोफेसर चंद्रिमा शाहा
अध्यक्ष
Prof. Chandrima Shaha
President

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03 February 2021

FOREWORD

Ever since the emergence of SARS-CoV-2 as the novel coronavirus that jumped into the humans in late 2019 from a yet uncharacterized zoonotic reservoir, there have been various speculations about the exact source of its transmission. The World Health Organization (WHO) has been seized with the task of conducting a thorough scientific investigation for which high powered teams of experts have been despatched to Wuhan, China from where the first case got detected. The COVID-19 disease caused by the virus has already led to substantial mortality, morbidity and societal disruption in almost all countries of the world, causing immense hardships and economic downfall.

Globally, the year 2020 witnessed a huge surge in infections across all age groups, with a substantial number of patients becoming life-threateningly ill leading to high mortality, particularly in Western Europe and the USA. Worldwide, over 100 million cases have already been detected as of January 28, 2021 with the US contributing about one-fourth and India and Brazil contributing one-tenth each to this whopping figure. Over two million people have already lost lives and this number is still increasing.

Due to its recent association with humans, the SARS-CoV-2 may not yet been fully adapted to the human host. This has led to the speculation that the virus may be evolving continuously towards even higher transmissibility. Recent reports indicate that the virus has accumulated novel mutations leading to patterns of genomic diversity, observed in various regions of the world. This may be an opportunity for the research community to follow the distribution and characterization of emerging mutations to track the course of the spread of the pandemic. The effort will help to identify sites putatively under selection as SARS-CoV-2 potentially adapts to its new human host.

The unifying hope of ending the global pandemic is the development of population-level herd immunity to halt the continuing cycles of infection and disease. Although no data exist to define the exact threshold necessary to achieve

herd immunity against COVID-19, modelling studies and exploration of data from similar viral diseases of the past suggests that more than 60% or even upto 80%, of the population may need to develop immunity for the viral replication rate to drop below 1, enabling a satisfactory level of disease control. Fortunately for India, both the infection numbers as well as the associated deaths have seen an impressive drop in recent weeks, igniting hope of an imminent natural herd immunity developing in parts of the country. Further, with the availability of vaccines to the Indian population, an overall sense of relief is being felt.

The pandemic has affected the scientific community in more ways than one, particularly the students. The loss of work that was halted during lockdown, reagents that expired, animals lost and continuity of research being interrupted has caused severe hardships to researchers across the spectrum. Due to the emergency situation, many scientists lost their jobs or had to shift to projects related to the pandemic; this naturally caused much anxiety and difficulty.

With the distribution of indigenously manufactured vaccines, we expect that the infection will go down soon and normal research activities can continue although much will be on the minds of the researchers looking back at the pandemic and anticipating the future.

The Executive council of the Indian National Science Academy decided to bring out a 'White paper' on COVID-19 immunity and vaccines. I wish to thank Prof Narinder K. Mehra FNA, former Dean of the All-India Institute of Medical Sciences, New Delhi for acceding to our request to undertake this important task. I am confident that the INSA Fellowship and other readers will find it a very educative document. My thanks are also due to Prof Ashok K. Singhvi FNA, Vice President of INSA for coordinating the activity on behalf of the Academy.

Chandrima Shaha

(Chandrima Shaha)

PREFACE

Challenges of a Deathly Pandemic

In the history of mankind, there have been several pandemics from the Justinian Plague of the 6th century to the Spanish Flu (HINI influenza) of 1918. The latter lasted for nearly two years, had three waves, 500 million people infected and over 50 million deaths. The people had become so sick of the continuing quarantine and social distancing measures that when they were first lifted, they rejoiced in the streets with uninhibited freedom as if let off from forced confinements. However, in the coming weeks, the second wave occurred and millions died.

The first two decades of the current century have already seen the emergence of three beta corona viruses – SARS epidemic of 2002 (916 deaths), MERS outbreak of 2012 (862 deaths) and now the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) of 2019, the last of the three causing pandemic outbreaks of COVID-19, a highly pathogenic respiratory disease. In a short span of time, it has become a rapidly spreading communicable disease, affecting well over 100 million people and global death toll amounting already to ~2.5 millions. Despite advancements in medical sciences, it is impossible to predict when the next infectious disease outbreak might occur. So, we need to be on full alert.

COVID-19 caused by the novel human beta coronavirus has attracted so much attention of the governments and people globally that no other disease has done in recent times. It is a stark reminder of the ongoing challenge of emerging and re-emerging infectious pathogens and the need for constant surveillance, prompt diagnosis, and robust research efforts to understand the basic biology of new organisms and human susceptibilities to them.

December 2019 will be remembered as a landmark month in human history when several cases of SARS-CoV-2 infection were first detected in the seafood wholesale market of Wuhan district of Hubei province of China. These were officially reported by China on January 7, 2020 and this aroused variable interest worldwide with most countries initially ignoring the novel infection. The first case outside of China was detected in

Thailand on January 13, 2020. India reported its first laboratory confirmed case at the ICMR's National Institute of Virology (NIV), Pune on January 30, 2020. This is also the date when the WHO declared it as a 'pandemic' and the public health emergency of international concern.

It is now clear that COVID-19 is a droplet infection, driven largely by human-to-human transmission, forcing public health officials to announce wearing face mask, frequent hand wash and practicing social distancing as the most effective ways to drive down the virus's reproductive numbers (known as Ro, and pronounced as R naught), meaning the average number of new infections generated by each infected person.

World over, the scientific community quickly got together in an exemplary international effort to develop effective countermeasures against the virus including robust diagnostics, novel treatment strategies and efficacious vaccines. There has been an unprecedented growth in scientific enquiry about various facets of the causative organism, underlying pathogenetic mechanisms, host immunity, epidemiology and clinical trials using new and repurposed drugs as well as scores of vaccines. The data generated so far has been particularly helpful in forecasting the disease outcome and for developing effective strategies of control.

An analysis done on the PubMed up until September 2, 2020 revealed that over 47,000 scientific publications on COVID-19 had already appeared in peer reviewed journals in a matter of just 8 months. This works out to nearly 5,875 papers per month which is 30 times higher than the nearly 196 papers per month for the other most awaited and the highly impactful subject of *Human Genome*. In fact, for human genome, it took 20 years to reach the figure of 47,000 publications in the subject.

This monogram is a comprehensive treatise with focus on host immunity, vaccines and ethical aspects of COVID-19 – the most significant pandemic that the human race has faced in recent times, leaving no country untouched. There are 8 chapters, written in easy-to-understand format and contributed by biomedical scientists of notable eminence having made significant contributions in diverse areas of medicine and biology.

My co-authors and I distributed the work amongst us such that chapters 1 to 5 dealing with general features of the pandemic to various aspects of the host immunity have been contributed by me and Gaurav Sharma. Chapter 6 on international COVID-19 vaccine development and clinical trials has been contributed by Sanjay Mahendale, chapter 7 covering the Indian status on vaccines by Narendra Arora and Gaurav Sharma, and the last chapter dealing with the ethical issues and regulatory challenges by Anant Bhan. The fundamental theme has been to capture the current state of the knowledge and express the same in simpler language without giving too much of hard data. The Indian National Science Academy wanted it to be a 'White paper' on COVID-19 immunity and vaccines.

Chapters 1 covers general features of the pandemic highlighting diverse areas beginning with the biology and pathogenesis of the SARS-CoV-2 virus, epidemiology and community spread of the COVID-19 disease giving key aspects of the SARS-CoV-2

virus, its transmissibility potential, other corona viruses afflicting man, challenges in laboratory diagnosis and the biological meaning of recurrent mutations.

The focus of chapter 2 is on disease immunopathogenesis including unique aspects of viral entry, clinical phenotypes and their prognostic kinetics, vulnerability of the elderly group and an introduction to the observed imbalanced host immunity. Chapter 3 deals in more details the immunopathology and immune correlates of COVID-19 vulnerability highlighting the unique feature of innate immunity hyperactivation and adaptive immune dysregulation during SARS-CoV-2 infection giving details on the current literature including the involvement of interferons and antibodies.

Chapter 4 discusses mechanisms underlying generation of long-term immunity to COVID-19 as a conundrum for vaccine design with special emphasis on facts related to herd immunity and whether Indians are more immune to COVID-19. Recent months have witnessed a remarkable downtrend in infection rates and fatality numbers in Indians, while the situation in rest of the world remains still alarming, particularly in western Europe, the UK, Brazil and USA. Although India stands at number two in terms of total infections, the case fatality rate in India has been the lowest at ~1.4%. Even more important is the very impressive recovery rate of >97% which is the highest in the world. In fact, the country's share of 'active cases' is the lowest at only 1.4% of the total infections, while France tops the list in terms of most active cases followed by UK and USA in that order.

When COVID-19 first struck the world in January last year, the biggest concern was that most of the low and low-middle income countries may have to face catastrophic consequences. However, the pandemic curve and especially the trends observed in the past nearly two months have thrown opposing results. The most plausible explanation that seems to explain this dichotomy is the '*Hygiene hypothesis'* discussed in chapter 4.

The broad-based immunity of the Indians driven as a result of the microbial load in the Indian sub-continent has been the most important factor. The cross-reactive antibodies generated would provide a level of protection to severe disease although direct proof for the same is a subject of future investigation. Otherwise what else can explain the unexpected low incidence of severe COVID-19 disease and of case fatalities in the much crowded and hygienically poor areas of the Dharavi slum of Mumbai and other similar areas elsewhere in the country.

More recently, a team of scientists from the National Institute of Biomedical Genomics in Kalyani, West Bengal, have found a biological reason for the slower spread of a mutant of coronavirus in Asia compared to the West. They explained how the spike protein mutation, D614G as well as a lung protein named *neutrophil elastase* helps the virus to spread faster from infected individuals. The level of this protein is modulated by its inhibitor, α-1 antitrypsin (AAT), deficiency of which leads to higher levels of neutrophil elastase in cells, causing faster spread of the virus. This deficiency is known to be much higher in Europe and America than among Asians. (journal of *Infection, Genetics and Evolution*, Feb 2021).

Chapter 5 discusses the potential role of host determined factors and genes that may determine the course of COVID-19 disease. Apart from the possible contribution of blood groups, the human leucocyte antigen (HLA) system with its known extraordinary diversity, particularly in the Indian population and of the killer immunoglobulin receptor molecules expressed on natural killer (NK) cells is important. Similarly, epigenetic factors that include environmental influences and life style are also important particularly in countries like India.

Chapter 6 by Sanjay Mahendale discusses the status on COVID-19 vaccine development internationally including phase I and II clinical trials and results of various platforms tried in this disease, some like mRNA, DNA based and vector-based vaccines for the first time. Further, there are details on vaccines that have either completed phase III trials or are in progress. The need is to have more than one safe, immunogenic and efficacious vaccine and protect the humanity from this difficult pandemic.

Chapter 7 by Narendra Arora with able assistance from Gaurav Sharma highlights the transformation of the Indian healthcare system and infrastructure needed for augmenting laboratory services and vaccine production capacity amid COVID-19 outbreak. Besides Covishield and Covaxin that are already in use, there are at least five more vaccines that are in advanced stages of testing and authorization.

The last chapter by Anant Bhan focusses on ethical issues and regulatory challenges, comprehensively covering all aspects of ethics. Special emphasis has been laid on the role of institutional ethics committees, community and public engagement, role and duties of vaccine manufacturers, challenges associated with emergency use authorization of vaccines and those with vaccine allocation and distribution in India.

Generally speaking, the presentation style of this book is easy with the text explanation supported by data in tabular and graphics format. Supportive references have been provided throughout the book making it a valuable publication on the current pandemic.

In the COVID-19 era, one witnessed unprecedented cooperation from the public in executing Government policies and advisories with the single most objective of defeating the virus. The vision of our honorable Prime Minister and the swiftness with which the health authorities swung into action has put India in a much safer position in just a matter of months. It is a matter of pride for every citizen to know that India is only the 5th country globally to have produced its own vaccines and distribute the same to several other countries including its immediate neighbors on humanitarian grounds.

It has been a pleasure for me to compile this 'White Paper' on COVID-19 on behalf on the Indian National Science Academy.

Prof. Narinder K. Mehra
On behalf of all co-authors

Feb 21, 2021

ACKNOWLEDGEMENTS

At the heat of the COVID-19 pandemic during August-September last year when the daily infection numbers and case fatalities were rising exponentially globally as well as in India, there was much scare and uncertainty about how the disease will finally get under control. The question was will the humanity see a repeat of history in time of COVID-19? I was contributing regular columns on SARS-CoV-2 immunity and genetics in Hindustan Times, and this was a great new experience for me.

The President of the Indian National Science Academy, Prof. Chandrima Shaha invited me to create a '*white paper*' focussing particularly on aspects of host immunity and vaccines on this disease. The idea was to bring out the monograph in a matter of few months. However, the amount and the scale at which the information has flown on the pandemic has been mind boggling with new knowledge emerging by each passing day.

I most profusely thank her and the INSA executive for giving me this responsibility, for it gave me the impetus to keep further updated on the remarkable discoveries happening on almost daily basis. I quickly gathered together a bunch of highly eminent people with remarkable track record to help me build the white paper. My sincere gratitude to all co-authors comprising Narendra K. Arora, Sanjay M. Mahendale, Anant Bhan and Gaurav Sharma, a former student for taking up important responsibilities in developing this unique compendium.

My special thanks are due to Prof. Ashok K. Singhvi, Vice-President of INSA for providing the much needed coordination and constant encouragement. Mr S. Karthikeyan deserves special mention for facilitating interaction and discussion sessions between the authors. Finally, our appreciation to Ms. Manisha Shrivastava and members of her publishing team of Angkor Publishers (P) Ltd in particular, Mr Bhagwan Das for completing the task elegantly and in time for release.

My co-authors and I derived the inspiration for preparing this White paper from none other than our Hon Prime Minister whose exemplary vision and foresight played a dominant role in saving the humanity from this deathly pandemic. I also

wish to place on record our deepest sense of appreciation for the Hon Minister for Health and Family Welfare, and Science and Technology, Government of India, Dr Harsh Vardhan for his unique style of mentoring that propelled India into *Atam Nirbharta* in her fight against COVID.

I sincerely hope that this work will go a long way in encouraging young minds to the fascinating field of viral immunology and vaccinology. This monograph has been prepared for public outreach and education on specific aspects of covid-19. Due acknowledgement to all the literature used in its preparation has been made. The publication is open access and has no commercial value or commercial interests whatsoever.

Narinder K. Mehra

ACRONYMS AND ABBREVIATIONS

Term/Abbreviation	Meaning
ATMANIRBHAR BHARAT	Self-reliant India
ACE-2 receptor	Angiotensin-converting enzyme-2 present primarily on type II alveolar cells of the human lungs.
ARDS	Acute respiratory distress syndrome
BSL-2/3 Facility	Bio-safety laboratory at level two or three
CAR-T cells	Chimeric antigen receptor transduced T cells
CEPI	Coalition for Epidemic Preparedness Innovations
CoV	Coronavirus
COVID-19	The WHO named the disease caused by SARS-CoV-2 infection as COVID-19: Co (Corona), Vi (Virus), D (Disease), started in 2019
Case fatality rate or CFR	Number of total deaths as on date / number of total RT-PCR positive cases on that day.
CLIA	Chemiluminescence immunoassay
CP	Convalescent plasma
CT Value	Cycle threshold value observed during the RT-PCR test. Although not an accurate measure of the viral load, a lower CT value generally denotes high viral content of the sample. Consequently, value on the higher side represents low viral content. Most laboratories treat a value above 32 as 'negative'.
Cytokines	A large group of proteins, peptides or glycoproteins which are secreted by specific cells, primarily immune system. These molecules act as signaling molecules for mediating and regulating immunity, inflammation and hematopoiesis
DAMPs	Damage associated molecular patterns
ELISA	Enzyme linked immuno-sorbent assay
GISAID	Global initiative on sharing all influenza data
GMCSF	Granulocyte monocyte colony stimulating factor
GWAS	Genome wide association studies
HCWs	Health care workers
HLA	Human leukocyte antigen
ICMR	Indian Council of Medical Research, a body created by the Ministry of Health and Family Welfare, Govt of India for the promotion of medical research in frontiers areas of human health

Term/Abbreviation	Meaning
ICTV	International Committee of Taxonomy of viruses, established with the task of naming novel viruses affecting human health. The committee named the novel coronavirus that originated from Wuhan China as SARS-CoV-2 due to its relatedness to the 2002 SARS coronavirus
IL-6	Interleukin-6
ILI	Influenza like illness
Interferons (IFNs)	Interferons are a group of signaling proteins released by host cells in response to viral infection, causing nearby cells to heighten their anti-viral defenses.
IFN-γ	Interferon-gamma
Immunocompromised	A broad term which means that the immune system is weaker than expected and not functioning properly
Immunity	The capacity to recognize and tolerate whatever belongs to the self, and to recognize and reject what is foreign (non-self)
Immunogenicity	Ability of the viral proteins to generate immune response in man, measured in terms of antibody formation and their titers, and T-cell mediated immunity
Isolation	Separation of a person with confirmed infection or contagious disease from the healthy and non-infected persons with the primary aim of interrupting transmission
Infection fatality rate	Number of total deaths as on date / number of total calculated cases as on that date.
Lockdown	A restriction policy for people or community to stay where they are, usually due to specific risks to themselves or to others if they can move and interact freely.
MCP-1	Monocyte chemo attractant protein-1
MERS	Middle East respiratory syndrome
MERS-CoV	Middle East respiratory syndrome coronavirus
MHC	Major histocompatibility complex
MHFW	Ministry of Health and Family Welfare
MIP-1α	Macrophage inflammatory protein 1 alpha
Neutralizing antibodies	Development of high titer IgG antibodies with ability to neutralize viral proteins.
NIV	National Institute of Virology, an ICMR supported institute located in Pune, Maharashtra
NGOs	Non-governmental organizations
ORF	Open Reading Frame
PAMPs	Pathogen associated molecular patterns
Pathogenicity	Refers to ability of the invading organism to cause disease in humans. This is different from infectivity which refers to potential of the foreign organism to infect humans
PPE	Personal protection equipment
Pneumonia	Infection that inflames air sacs in one or both lungs, which may fill with fluid.
PRRs	Pattern recognition receptors
Quarantine	Movement restriction of persons presumed to have been exposed to a highly transmissible infectious agent, even though the person himself/herself may not yet been infected as determined by the laboratory testing or been still be in the incubation period.

Term/Abbreviation	Meaning
R0	Rnaught, meaning the average number of new infections generated by each infected person
RBD	Receptor binding domain in the spike protein of the virus
RT-PCR	Real time polymerase chain reaction
SARI	Severe acute respiratory illness
SARS	Severe acute respiratory syndrome
SARS-CoV	Severe acute respiratory syndrome coronavirus
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SIRS	Systemic inflammatory response syndrome
Social Distancing	Asking people to maintain a distance of at least six feet between them so as to halt or restrict the transmission of an infectious agent and thus prevent person-to-person spread of the disease.
Spike Protein	The spike (S) protein of SARS-CoV-2, which plays a key role in the ACE-2 receptor recognition/binding and cell membrane fusion process
TLR	Toll like receptors
TMPRSS2	Transmembrane protease, serine 2 is an enzyme that in humans is encoded by the TMPRSS2 gene
Transmissibility	Refers to the ability of the virus to transmit disease from person-to person at the population level
TNF-α	Tumor necrosis factor-alpha
Vaccine	A biological (immunogenic) preparation that provides active acquired immunity to a particular infectious disease.
VTM	Viral transport medium, needed for collection of the nasopharyngeal sample for transport and testing.
Vaccination	A simple, safe, and effective way of protecting people against harmful diseases, before they come into contact with them through vaccines
WHO	World Health Organization with its headquarters located in Geneva, Switzerland

Some Useful Websites:

1. https://www.mohfw.gov.in/
2. https://www.mygov.in/covid-19/
3. https://covid19.who.int/
4. https://www.icmr.gov.in/
5. https://www.aarogyasetu.gov.in/
6. https://www.cowin.gov.in/home
7. https://www.worldometers.info/coronavirus/
8. https://cdsco.gov.in/opencms/opencms/en/Home/
9. https://nabl-india.org
10. http://dashboard.dbtindia.gov.in

Articles on COVID-19 immunity and vaccines

published by Professor N.K. MEHRA, FNA in Hindustan Times*

HT-1: April 1, 2020:	Can India be an outlier in the spread of COVID-19? (This article had a special video commentary by Mr. Shekhar Gupta, Editor Print)
HT-2: April 10, 2020:	COVID-19: India has done well till now, but lifting the lockdown is not advisable
HT-3: May 21, 2020:	COVID-19: The science behind India's trajectory.
HT-4: June 17, 2020:	Many countries saw a dip in cases. There are lessons.
HT-5: July 24, 2020:	The twin tales of vanishing antibodies, robust T-cells
HT-6: August 12, 2020:	COVID-19: Decoding the global search for a vaccine.
HT-7: September 22, 2020:	The scientific enquiry on COVID-19 immunity.
HT-8: October 7, 2020:	Alternate medical tools against COVID-19.
HT-9: October 28, 2020:	Genes may decide the coronavirus disease path.
HT-10: November 17, 2020:	COVID-19 vaccine: Room for cautious optimism
HT-11: December 31, 2020:	Mutant strains and the need for strict controls.

*These are available on request at narin.mehra@gmail.com

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EXECUTIVE SUMMARY

General

- The mankind has seen several pandemics beginning with the Justinian Plague of 6th century to the Spanish Flu (HINI influenza) of 1918. The current century has already seen outbreaks with three beta coronaviruses - SARS epidemic of 2002 (case fatality rate 10-11%), MERS outbreak of 2012 (CFR 34.5%) and now the SARS-CoV-2 of 2019 with its very high transmissibility, but much lower case fatality rate (~2.2%).
- China detected the first case of COVID-19 on January 7, 2020 and traced it to the seafood wholesale market of Wuhan district of Hubei province.
- India reported its first laboratory confirmed case at the ICMR's National Institute of Virology, Pune on January 30, 2020. This is also the date when the WHO declared it as a 'pandemic' and the public health emergency of international concern.
- COVID-19 is a droplet infection, driven largely by human-to-human transmission. A mutation in the spike protein "S' of the virus, D614G has been linked to increased transmission and infectivity.
- The virus causes severe inflammation and damage to endothelial cells of lungs and other tissues, has a mean incubation period of 5.2 days, mean time to symptoms of 5 days and mean time to death of 14 days.
- Diagnostic testing options include the gold standard RT-PCR and a less sensitive rapid antigen test. Antibody determination for IgG/IgM or total antibody is also done for sero-surveillance or to determine exposure and immunity levels.
- Generally speaking, low and low-middle income countries including India have experienced significantly reduced disease severity and much lower CFR as compared to the more affluent nations. The most plausible explanation that seems to explain this dichotomy is the '*Hygiene hypothesis*' and the prevalence of broad-based immunity in the background of high microbial load in these countries. It is possible that cross-reactive antibodies provide a level of protection to severe disease although direct proof for the same is a subject of future investigation.

Host Immunity

- Immune patterns are closely associated with clinical disease progression.
- Majority of the COVID-19 cases (~80% or more) are either asymptomatic or develop mild disease. Of these, ~30% are able to develop neutralizing antibodies against the virus and stay healthy. Another 50% develop mild or low moderate symptoms lasting for 3-4 days, eventually becoming immune and recover with no mortality. All of these people are advised to stay in home quarantine.
- Only about 10% of the RT-PCR+ve subjects develop moderate to severe symptoms requiring hospitalization and perhaps oxygen support. Most recover after a variable period of 10-15 days, develop anti-COVID immunity and are discharged. However, approximately 15% of these patients gradually develop severe disease and may die.
- A very small number (3-5%) of test positive patients, particularly those with additional comorbidities or the elderly progress towards severe disease requiring ventilatory support in the intensive care unit and the death rate in this group is up to 50%.
- COVID patients have lymphopenia with an almost 20% drop in their lymphocyte numbers in severe cases. They also show marked reduction in CD4+ T, CD8+ T, and NK cell numbers as compared to the mild cases.
- Severe COVID-19 patients exhibit elevated levels of several cytokines, particularly of IL-1β, IL-6 and IL-10, leading to 'cytokine storm' and inflammation-induced multiple organ dysfunction. IL-6 is important in inducing cytokine storm and inflammation.
- Patients with severe disease are characterized by B-cell activation and an exaggerated IgG response, indicative of a poor outcome.
- COVID-19 is a case of innate immune hyperactivation on one hand and of adaptive immune dysregulation on the other. The former phenomenon is responsible for driving acute lung injury, while the latter leads to increased risk of viral reactivation.
- The high disease severity and mortality observed in the very senior citizens above age 80, for example in Italy is due to the age-related waning of adaptive immune function, also known as '*immunosenescence*', characterized by a loss of T-cell clonal diversity and a contraction of naive T-cells with proliferative capacity.
- A generally protective role of interferons (type 1) has been seen in COVID-19 because auto-antibodies against these molecules could prime the immune system towards redundancy leading to onset of critical disease. This occurs mostly in male patients above 65 years of age.

- Preponderance of males with severe COVID-19 disease globally and with higher fatality rate could also be genetic. Women generally have higher rates of autoimmune disease; so one hypothesis could be genetic, due to X-linked recessive trait. Women with two X-chromosomes are protected, while men with one are not.
- SARS-CoV-2 specific antibodies decline after a period of 4 to 6 months. Nevertheless, depleting antibodies do not signify that the person doesn't have immunity anymore since an all protective army of T-cells (CD4 and CD8) can stay in the circulation for years.
- Long-lasting immunological memory is a hallmark of an effective and preventive vaccine strategy. In this context, there has been scanty evidence of re-infection with COVID-19.
- On the question of Herd immunity, most models estimate that at least 70% or more of the population must become immune to the virus, which is possible only through community vaccination drives.
- Genes and host determined immune response factors could decide the clinical course of coronavirus disease. This includes blood groups, HLA allelic phenotypes, toll-like receptor genes, epigenetic factors and others.

Vaccines

- Significant advancements have happened in the area of bio-medical technology and instrumentation over the last few decades which have resulted in reduction in time required for candidate vaccine development.
- COVID-19 vaccine has been an urgent public health requirement for which, novel approaches have been adopted including concurrent animal and human evaluation, designing phase IIb trials with early efficacy end points and focusing on relatively new platforms like vector based vaccines and DNA/ mRNA/ protein/ subunit vaccines.
- Further, a more meaningful engagement and flexible as well as adaptive approach by Ethics Committees and Regulatory Bodies in providing timely approvals for human clinical trials as well as product licensure eventually has been considered important for fast track COVID-19 vaccine development.
- Vaccine developers faced additional challenges such as undefined vaccine targets, high cost of manufacturing of the novel products, limited production capacity of traditional vaccine approaches, uncertainty about immune correlates of protection and, novel requirement of vaccine storage at ultra-low temperatures. Despite all these, progress on COVID-19 vaccine development has been truly unprecedented.
- Several constructs in the categories of inactivated, DNA, mRNA, non-replicating viral vectors, protein subunits, antigen presenting cells vaccines or previously

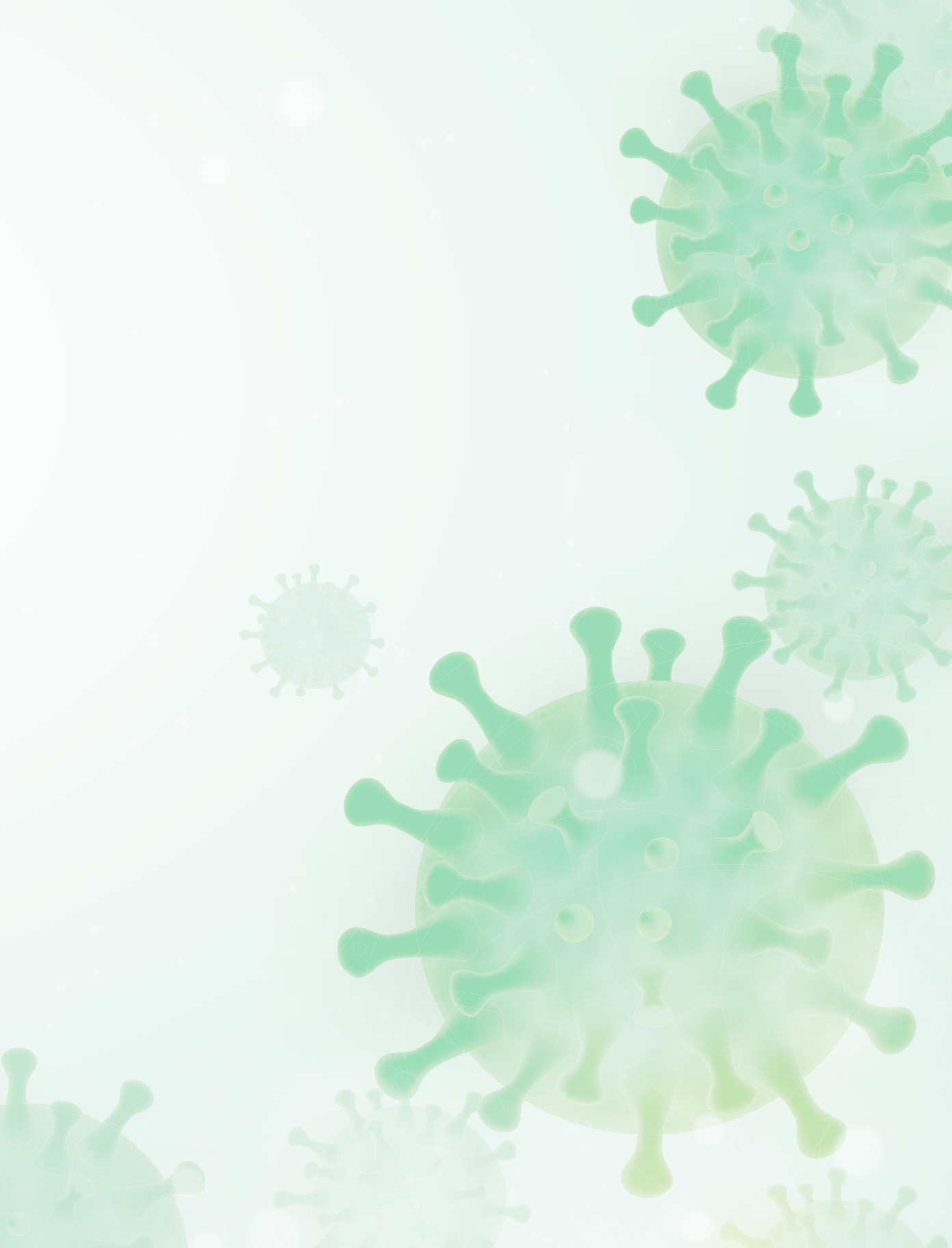
approved vaccines are in Phase I and II clinical trials globally. Many of them have demonstrated promise in Phase I and II trials in terms of safety and immunogenicity and have advanced into phase III trials.

- Vaccines produced by i) Moderna (Cambridge, Massachusetts), ii) University of Oxford and Astra Zeneca (Cambridge, UK) and iii) BioNTech (Mainz, Germany) in collaboration with Pfizer has entered Phase III trials in humans a few months back. In addition, the adenovirus vector based Russian vaccine Sputnik V and the one by Johnson and Johnson's Ad26.CoV2.S are the other front runners along with six Chinese vaccines. Results on majority of the ongoing clinical trials are expected in early 2021 when the picture about vaccine constructs with high efficacy will be more clear.
- In addition to their ongoing evaluation in Phase III clinical trials, many of the vaccine manufacturers have secured '*emergency use authorization*' in several countries that have rolled out their own national COVID-19 immunization programs in a systematic manner beginning with vaccination of frontline workers including health care workers in the first phase, senior citizens and older adults with co-morbidities in the second phase and the general people in the third phase.
- The Government of India played a highly commendable role by supporting several vaccine development projects, creating clinical trial sites all over the country and facilitating Phase I, II and III vaccine trials in the country. There are 8-10 potential indigenously developed Indian COVID-19 vaccines in the pipeline, some of which are likely to get into clinical evaluation in early 2021.
- Although currently COVISHIELD (Astra Zeneca – University of Oxford – Serum Institute of India) and COVAXIN (Bharat Biotech – Indian Council of Medical Research) have been rolled out in India, there is a distinct possibility that trials on Sputnik V, Johnson and Johnson's adenovirus vector vaccine will soon be allowed along with their roll out for the Indian population.
- Effective roll out and optimum coverage of vaccines would require a lot of ground work such as deciding priority population categories for vaccination, creating line listing of potential recipients, building stocks of the nationally approved vaccines and training staff that can be fully involved in vaccination exercise.
- It is equally important to examine issues around vaccine hesitancy and barriers to vaccine uptake. For the vaccination program to succeed, it is important to ensure adequate communication regarding vaccine safety, effectiveness, possible adverse reactions, follow-up care options and equitable access to vaccines. Creating awareness in all sections of the society through effective community engagement is desirable.
- India has committed a major contribution of vaccines to the global COVAX facility and shared almost 20 million doses with 18 countries under bilateral arrangements.

- COVID-19 pandemic led India to an unprecedented strengthening of her health system infrastructure within a short period of 10-12 months and building on the existing immunization infrastructure and building a *de novo* IT platform for roll out of the vaccines.
- India's focus on ATMANIRBHAR BHARAT along with financial support from state and academic partnership has realized the enormous entrepreneur capacity of Indians. Many of the critical items required for diagnosis and management of COVID-19 are now manufactured locally. India transformed from a net importer of several medical supplies and equipment to a highly acclaimed exporter.
- The political leadership of the country provided great encouragement by investing liberally in the health sector and this will prove to be a great step towards improving the economy of the country and international diplomacy.

Ethical Issues

- The ethical issues involved in vaccine development include the informed consent process, privacy and confidentiality, requirements around transparency and accountability, site/selection, role of ethics committees as well as mechanisms related to emergency use authorization.
- There are also issues related to fair vaccine allocation, ethical facets of mandatory vaccination, use of biometric Ids, vaccine 'passports', infrastructure provisioning, adverse events and vaccine hesitancy.



CHAPTER 1

EMERGENCE OF COVID-19 PANDEMIC

In the history of mankind, there have been several pandemics beginning from the Justinian Plague of 6th century to the Spanish Flu (H1N1 influenza) of 1918. The 21st century remarkably has already seen three coronavirus outbreaks in its first two decades (**Fig. 1**). These include the Severe Acute Respiratory Syndrome (SARS) epidemic of 2002 with 8,422 cases and 916 deaths (fatality rate of ~10-11%), MERS outbreak of 2012 with 2,494 cases and 862 deaths (fatality rate of 34.5%) and the current

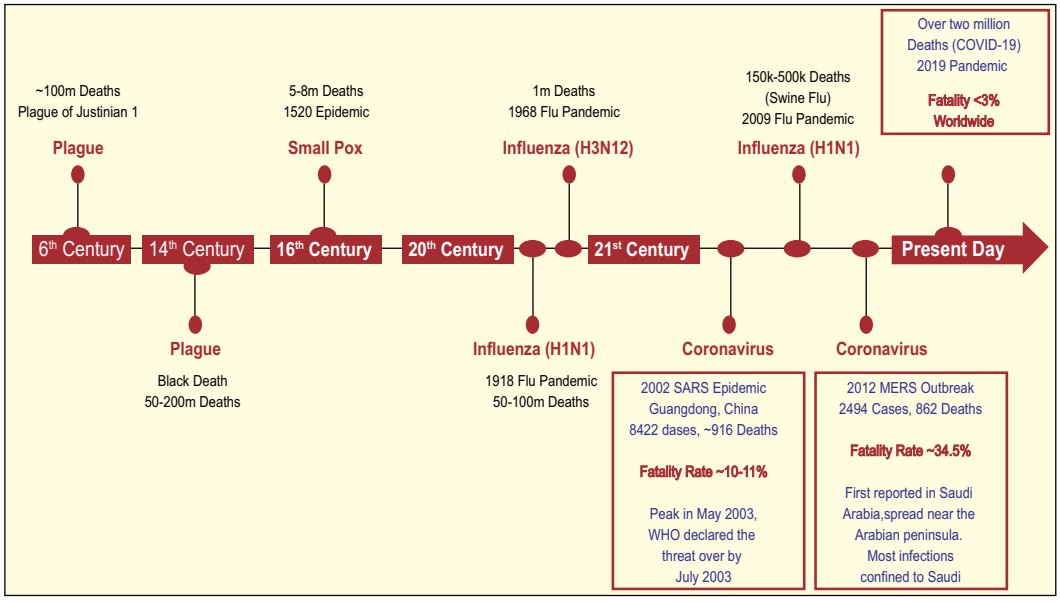


Fig. 1: *An overview of the history of pandemics experienced by humans over centuries. In recent times, the most prominent of these is the 1918 Flu pandemic caused by the H1N1 influenza virus that caused 50–100 million deaths.*

coronavirus pandemic that first got noticed towards the end of 2019 in China through a possible zoonotic (animal to human) route (**Table 1**). Despite advancements in medical sciences, it is impossible to predict when the next infectious disease outbreak will take place. So, the health authorities need to be on full alert the world over.

The World Health Organization (WHO) initially declared it as an outbreak of febrile respiratory illness of unknown etiology. The causative agent was isolated from the lungs of three infected individuals and labelled as "novel coronavirus" that must have entered humans as a jump species from Bats through an intermediary animal species. Soon, it became clear that the infection is spread from human-to-human transmission through droplets of an infected individual by coughing and sneezing or through prolonged contact with an infected person or surface. It was much more contagious than the earlier two reported corona viruses and was labelled as the 'novel coronavirus'. The International committee of Taxonomy of Viruses (ICTV) named it as SARS-CoV-2 due to its relatedness to the 2002 SARS coronavirus. The WHO named the disease caused by it as COVID-19: Co (Corona), Vi (Virus), D (Disease) that started in 2019.

Table 1: Some important clinical and epidemiological details of the three coronavirus infections experienced in the first two decades of the 21st century. In a short span of one year, the SARS-CoV-2 has already infected ~102 million people worldwide, highlighting higher transmissibility of this virus as compared to the earlier two

Viruses	Key clinical and epidemiological details							
	Dates	First case	Spread (countries)	Incubation (days)	Transmissibility	No. of infections	No. of Deaths	Mortality Rate
SARS	November 2002-July 2003	China	26	2-10 (7)	Moderate	8422	916	~10.1%
MERS	September 2012-till date	Saudi Arabia	27	2-10 (5.5)	Low	2494	862	~34.5%
SARS-COV2	December 2 019-January 30, 2021	China	~213	2-14 (5.2)	High	~102 million	~2.2 million	~2.2%

First Case Reported

COVID-19 first appeared in the seafood wholesale market of Wuhan district of Hubei Province of China in early December 2019. The Chinese Government alerted the World Health Organization of several cases of coronavirus disease on December 31. The first real case of COVID-19 was reported by China on January 7, 2020 and the first death on January 11. Though most countries initially ignored this novel infection, the Indian health authorities including the Indian council of Medical Research (ICMR) became active almost immediately reporting the first laboratory confirmed case at the National Institute of Virology (NIV), Pune on January 30, 2020 (details below). India reported its first death of COVID-19 on March 12, exactly two months later than in China (**Table 2**). In a short span of time, COVID-19 has become a rapidly spreading

communicable disease and on January 30, 2020, the WHO declared it as the "Public Health Emergency of International Concern".

Table 2: A summary of the initial key events of COVID-19 pandemic in India. China reported its first case a month earlier and imposed complete lockdown of only the Wuhan District two months earlier than the lockdown in India. However, in India it was countrywide and the public participation was commendable, a great feat achieved by the country with remarkable diversity of its people

Event	India	China
First Case	January 30, 2020	December 31, 2019
First Death	March 12, 2020	January 09, 2020
Lock down	March 25, 2020	January 23, 2020 (in Wuhan)
Cluster identification (Delhi)	March 31, 2020	
100 confirmed deaths	April 05, 2020	
Second lock down	May 01-17, 2020	
50,000 confirmed cases	May 07, 2020	
Third lock down	May 17-31, 2020	
1,00,000 confirmed cases	May 19, 2020	
10.7 million cases	January 30, 2021	

It is now clear that COVID-19 is a droplet mediated infection, driven largely by human-to-human transmission, forcing public health officials to sensitize the public on COVID appropriate behaviour during the pandemic. Announcements and advisories were issued asking everyone to wear face mask, do frequent hand wash and practice social distancing of a minimum of two meters as the most effective ways to drive down the virus's reproductive numbers. The latter are known as Ro, and pronounced as R naught, meaning the average number of new infections generated by each infected person.

Soon it was clear that SARS-CoV-2 is highly infectious, but comparatively less lethal with the current global case fatality rate of ~2.2%. As per the World Health Organization, there is a considerable disparity both in infection numbers and death rates among nations with the highest rates of fatalities recorded in Europe, North America and Brazil, and the least in Africa and the Indian sub-continent.

The current pandemic is unique in many ways. It is caused by a new virus that leads to the generation of dichotomous host immunity as explained later in this book. It is a unique case of innate immunity (pre-existing, ready to attack) in over drive mode leading to a 'cytokine storm' in the more severe cases. On the other hand, the adaptive arm of the immune response, which is more potent, long-lasting and much-desired remains suppressed. The challenge at hand was to quickly and efficiently vaccinate the global population while continuing developing novel therapeutic strategies for maintaining an optimal balance between the two, so as to halt viral replication and promote the destruction of virus-infected cells.

In a matter of months since the onset of COVID-19, there has been an unprecedented explosion of scientific enquiry on all facets of the disease, particularly in relation to the population and country-wise prevalence, host immunity, clinical profile and spectrum, control measures, treatment strategies and finally the recently evolved vaccination approaches as well as their efficacy. The data generated so far has been particularly helpful in forecasting the disease outcome and for developing effective strategies of control.

Growing Infections and Fatalities the World Over

As of January 30, 2021, more than 100 million infections (101,993,568) have already been recorded worldwide. The three leading countries include USA over 25 million (25,890,758), India over 10 million (10,720,048) and Brazil with over 9 million (9,118,513) cases. In terms of percent global share, every fourth infected person is from the US and every tenth from India. The virus has spread to 215 countries/territories with variable rates of spread and is still rising, particularly in Western Europe, USA, Brazil and Russia.

The world has already recorded nearly 2.2 million (2,203,696) deaths, making the case fatality rate of ~2.2%. Most number of deaths have been reported by the US, followed by Brazil and Mexico, with India falling to 4th place (**Table 3**).

Globally, India accounts for 10.5% of the total infections and only 6.9% of the total fatalities attributed to the virus. Its case fatality rate (CFR) is among the

Table 3: Total number of COVID-19 infections and deaths in top 10 countries (data as of Jan 30, 2021). The case fatality rate (CFR) shows Mexico leading with 8.5% with India at 1.4% at the lowest. Interestingly, India's 1.4% CFR has remained constant throughout the pandemic

Total Cases (n=101,993,568)			Case Fatality Rate (CFR)*		Total Deaths (n=2,203,696)		
Country	**Cases**	**% share**	**Country**	**%**	**Country**	**Deaths**	**% share**
USA	25,890,758	25.4%	Mexico	8.5%	USA	435,892	19.8%
India	**10,720,048**	**10.5%**	Italy	3.5%	Brazil	222,666	10.1%
Brazil	9,118,513	8.9%	UK	2.8%	Mexico	155,145	7.0%
UK	3,783,593	3.7%	Brazil	2.5%	**India**	**154,010**	**6.9%**
Russia	3,771,514	3.7%	France	2.4%	UK	104,572	4.7%
France	3,212,613	3.1%	Germany	2.4%	Italy	87,858	4.0%
Spain	2,743,119	2.7%	Spain	2.1%	France	75,765	3.4%
Italy	2,529,070	2.5%	Russia	1.9%	Russia	71,054	3.2%
Germany	2,207,393	2.2%	USA	1.7%	Spain	58,319	2.6%
Columbia	2,077,633	2.0%	**India**	**1.4%**	Iran	57,807	2.6%
Mexico	1,825,519	1.8%			Germany	53,284	2.4%

*CFR is the number of deaths divided by the number of infections of a particular country.

lowest in the world at 1.4% and this has remained constant ever since the early months of the pandemic. Mexico leads the table with the highest CFR of 8.5%, followed by Italy (3.5%) and the UK (2.8%). The US situation reporting by far the highest number daily deaths and infections has been scary, and the trend has continued since the middle of last year.

Record Number of Global Daily Deaths

As the month of January 2021 comes to a close, the global daily deaths are still rising, perhaps at the fastest rate yet. The seven-day average of daily deaths also known as the death rate is at 14,244 fatalities a day as of January 2021 – the highest ever recorded globally. In fact it is perhaps twice of what was seen during the first global wave of infections when the average daily death rate was ~7000 during late April, 2020.

After a brief respite seen during the later months of 2020, when it appeared that the global deaths may be dropping, the number has again started to rise. This may be due to an unexpected surge in additional cases caused by the new variants of the virus in UK and USA. For example, a total of 17,116 deaths were reported across the world on January 29, 2021, nearly half of these (49%) were the residents of just five countries, namely USA (24%), UK (9%), Brazil (7%), Germany (5%) and Russia (>4%).

India on the other hand, has consistently recorded an average of a little over 13,000 infections and 145 deaths attributable to COVID-19 infection during the whole month of January 2021.

Situation in India

Globally, the daily record of new cases, deaths, recovered and active cases has been meticulously maintained in the World COVID meter. An important component here is to look at the 'Growth Factor' (GF), which provides information by which a quantity multiplies itself over time. The formula used is the number of new cases every day/ the number of new cases on the previous day. For example, a quantity growing by 7% every period (in this case daily) has a growth factor of 1.07. A GF above 1 indicates an increase and if it remains constantly above 1, it could signal exponential growth.

Similarly, a record on the 'doubling time' of the coronavirus cases provides a clear picture about the growth trajectory of the pandemic globally and region wise. Globally, the first case was reported on January 10, 2020 and it took 67 days for the number to go up to 100,000. However, for the second one lakh, it took only 11 days, the 3rd in 4 days, 4th in 3 days, 5th in 2.5 days, 6th in 2 days, 7th in 2 days and subsequent in 1.5 days.

The pandemic in India started at least a month later recording its first case on January 30, 2020. By 10th April, the number had reached 7,600 and by 18th April, there were already 14,352 cases. This represents a doubling time of 8 days (**Table 4**).

Table 4: Trajectory of COVID-19 cases in India (Data of last four winter months)

Date	New Cases	Total Cases	Total Deaths	Active Cases
January 30, 2021	NA	10,720,048	154,010	NA
January 20, 2021	15,277	10,611,719	152,906	193,650
January 10, 2021	16,085	10,467,431	151,198	224,103
January 01, 2021	17,080	10,303,409	149,205	252,275
December 20, 2020	24,589	10,056,248	145,843	305,015
December 10, 2020	34,666	9,796,992	142,222	364,582
December 01, 2020	36,456	9,499,710	138,159	429,753
November 20, 2020	46,288	9,050,613	132,764	441,952
November 10, 2020	44,679	8,635,754	127,615	NA
November 01, 2020	46,441	8,229,322	122,642	563,775
October 20, 2020	54,422	7,649,158	115,950	740,658
October 10, 2020	74,535	7,051,543	108,371	868,309
October 01, 2020	81,693	6,391960	99,804	NA

One Year After the First Case

On January 30, 2021, India completed one full year of the pandemic recovering over one million infections. The good news, however has been that the numbers have started to decline in most states with each passing day. The cumulative number of deaths have also been falling consistently over the past several weeks.

It was exactly one year earlier on January 30, 2020 that the first case of COVID-19 was recorded in India, of a 21 year old female medical student from the state of Kerala who had returned from Wuhan, China, from where the virus is believed to have originated first. The young woman was immediately shifted to a treatment facility where she stayed for 28 days till recovery. Subsequently, two more students returned from Wuhan tested positive and had to be similarly treated.

These three cases marked the beginning of the pandemic in India. Incidentally, while the surge in numbers has subsided considerably all over India, the pandemic still continues to rage in Kerala. On January 30, 2021, Kerala accounted for 48% of the new 13,064 cases that the country recorded on that day.

As per the worldometer.info of January 30, 2021, five countries have recorded more than one lakh deaths from the infection so far. The US has registered by far the most deaths with the toll of nearly 4.4 lakhs. This is followed by Brazil with over 2.2 lakh deaths, Mexico with 1.55 Lakh, India 1.54 and the UK with 1.03 lakh deaths (**Fig. 2**). Surprisingly, Mexico recorded over 1500 deaths in one day going past India's total recorded deaths from the virus so far.

India remains the only country with a single peak and death rate showing a consistent drop for over a month. This is in contrast to the situation in other four countries, all of which are recording a second peak in the number of COVID related fatalities.

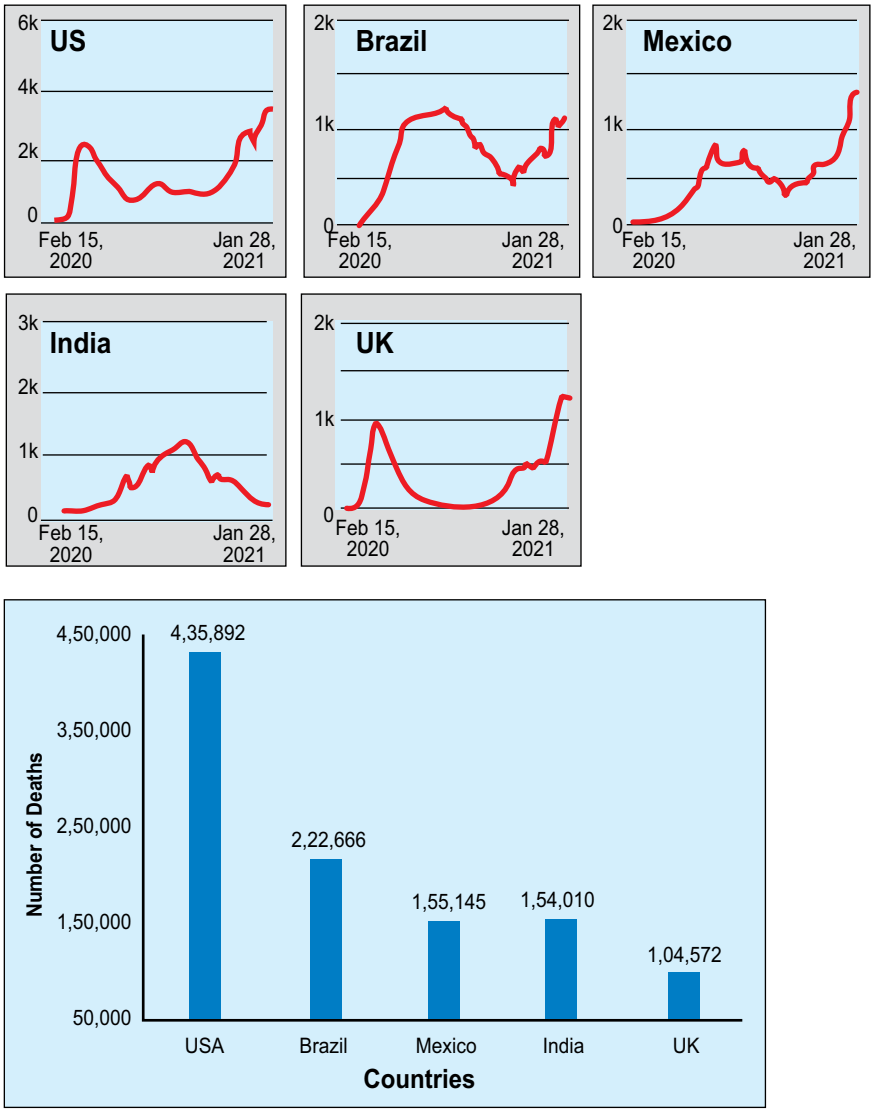


Fig. 2: *Five countries with number of deaths exceeding 100,000 as on Jan 30, 2021. a) graph represents a 7-day average number of daily deaths beginning February 15, 2020 until Jan 28, 2021, b) total number of deaths recorded from the very beginning of the pandemic until Jan 30, 2021 (Source: Worldometers.info).*

India led the world in 'daily COVID case counts' from late August till October last year, clocking a record 98,000 cases on September 16. In terms of daily numbers, It has fallen to the eighth spot in January 2021 because the seven-day rolling average of daily cases now stands at around 13,500. This is significantly lower than the daily averages recorded by the US, Brazil, Russia, UK, France, Spain and Mexico.

Likewise, the test positivity rate in India has dropped significantly from ~25% in August-September 2020 to a mere 2 to 5% in January 2021 depending on the region. Further, the CT (cycle threshold) value of the RT-PCR test of those reported positive is around 28-30, suggesting an appreciable drop in the overall viral load in the country.

Government Announced Lock Downs: *Turning challenge into an opportunity*

After the highly successful '*Junta curfew*' announced by the Hon. Prime Minister on March 22, 2020, the Government of India imposed stringent curbs on activities and travel by promulgating the first lock-down on March 25, 2020 that lasted for 68 days with two extensions. Several ministries, departments, NGOs and self-help groups got together in managing the outbreak from the very beginning and the process helped in saving crucial time between decision making and execution.

The collaborative spirit witnessed between the scientific community and the medical professionals with the single most objective of saving human lives by creating the minimum infrastructure needed for the purpose has been unprecedented and never witnessed earlier. Augmenting health care infrastructure, adequate availability of hospital beds, trained manpower including doctors, nurses and other healthcare workers became a national challenge for India. The Make-in-India *mantra* provided by the honourable Prime Minister became the guiding principle for the Government to incentivise public health measures.

From conducting just ten diagnostic tests per day on January 30, India reached a figure of over 12 million tests per day by mid-December last year. This is the highest number of tests performed by a single country surpassing even the United States' efforts. India has already tested over 155 million samples as of mid-December 2020, which is a record of sorts considering the near hopeless situation at the beginning of the year.

Similar to the efforts on the laboratory diagnostics, our scientists, medical professionals, innovators and entrepreneurs in the industry, health planners and administrators acted in a highly coordinated manner to turn the pandemic challenge into an opportunity. The goal was how quickly to exit from the pandemic, keeping the morbidity and mortality to as low as possible.

Hospital Admissions, Treatment and Indigenous Vaccine Development

The most important challenge for the policy planners was to prepare a model for providing hospital admissions and efficient treatment to those getting infected and thus save lives in the short term. This was a gigantic task considering the huge gap that existed in the demand and supply, and the general inadequacies in the health infrastructure in the hospitals. The country hardly manufactured her own

ventilators, personal protection equipment (PPE kits), adequate oxygen supplies and other infrastructure needed for the care of the sick in hospitals. It is a matter of pride for us that today India stands fully self-reliant in all of these needs and is even in a position to export to other countries. This by no means is a small achievement.

At the height of the pandemic, it was clear to all that vaccination was the only way to achieve 'herd immunity' and save the public from dreadful impact of the pandemic. Again it is a matter of pride for India that the industry, entrepreneurs and the scientific community got together in the spirit of unmatched collaboration and have already created two very effective vaccines, both manufactured in India. Further, there are at least four more vaccines against the coronavirus in various stages of testing and development in the country. All of these together will fulfil the goal of vaccinating everyone and thus help in achieving community immunity against the novel virus at the earliest.

It may be mentioned that India is only the 5th country in the world (the other four being USA, China, Russia and the UK) to manufacture their own in-house vaccines. January 16, 2021 became the landmark day in history when the world's largest inoculation drive began in India with the Pune based Serum Institute of India (SII) manufactured Covishield (developed by the University of Oxford and British-Swedish Pharma company AstraZeneca) and the Bharat Biotech's indigenously produced Covaxin. Most importantly, India is also supplying vaccines to its neighbours and to several other countries.

SARS-COV-2*: Key virological features*

Coronaviruses are a diverse group of single-stranded positive-sense RNA viruses with a wide range of vertebrate hosts[1]. The SARS-CoV-2 is round or elliptical in shape with a diameter of approximately 60-140nm (**Fig. 3**). The M protein is responsible for the transmembrane transport of nutrients, the bud release and the formation of envelope. The S (spike) protein attaches to the host receptors in man, chiefly the angiotensin converting enzyme 2 (ACE 2) through two subunits, S-1 and S-2. The former determines the virus host range and cellular tropism by the receptor binding domains (RBD). The S-2, on the other hand, mediates the virus cell membrane fusion by heptad repeats – HR1 and HR2. Besides, there are 16 non-structural proteins, designated as nsp1 to nsp16.

Briefly, the viral biogenesis cycle includes TMPRSS2 mediated activation and cleavage of SARS-CoV-2 S glycoprotein to facilitate receptor (ACE2) binding on epithelial cells of the respiratory tract. This is followed by viral entry into the host cytosol and replication including sub genomic RNA synthesis and assembly. Mature virions utilize host machinery as these are transported to cell surface in the vesicles and are released by exocytosis.

It is now recognized that both, the earlier coronaviruses (SARS-CoV) and the current SARS-CoV2 utilize the receptor binding domain (RBD) for binding to the

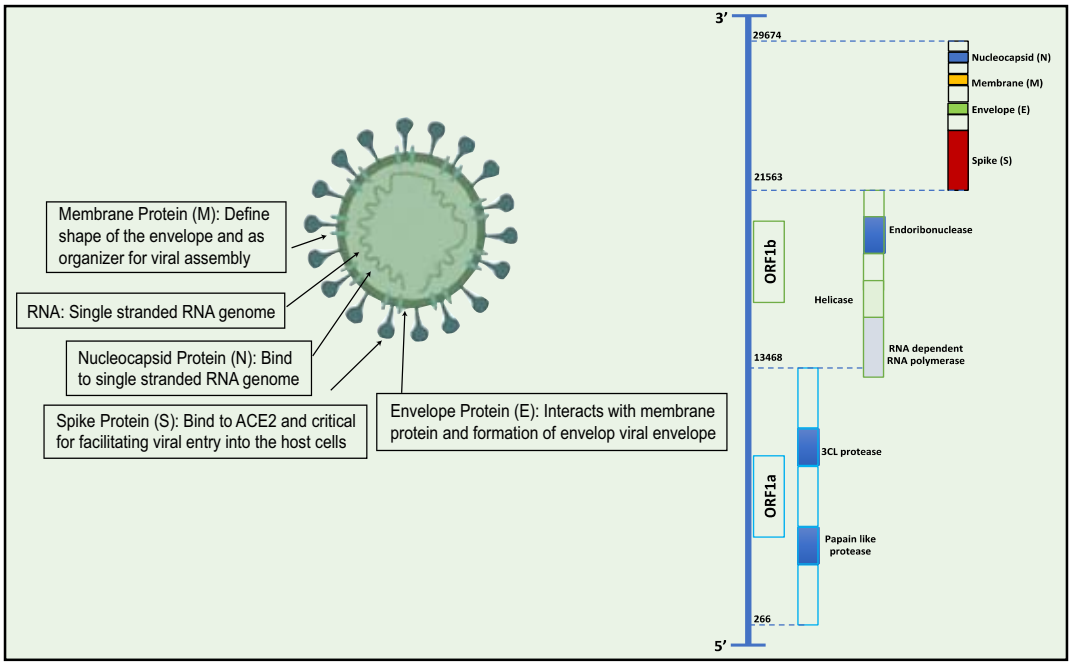


Fig. 3: *General Structure and genomic organization of SARS-CoV2. Left half of the figure represents a diagrammatic view of the virus with spikes making it resemble a crown, hence the name corona. The right half reflect simplified genomic organization of SARS-CoV2 which has single stranded RNA (ssRNA) genome (~30 kb). The open reading frame (ORF1) a/b encodes a polyprotein and other genomic regions are responsible for coding the four structural proteins: spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins plus several accessory proteins required in viral biogenesis cycle.*

ACE-2 receptor. Binding to the ACE2 receptor is a critical initial step for SARS-CoV to enter into target cells. Several studies have highlighted the important role of ACE2 in mediating entry of SARS-CoV-2. In vitro binding measurements have revealed that the SARS-CoV-2 RBD binds to ACE2 with an affinity in the low nanomolar range, indicating that the RBD is a key functional component within the Si subunit that is responsible for binding of SARS-CoV-2 by ACE2[2].

The conserved non-RBD regions in the spike protein (S2 subunit) are the potential targets for cross-reactive antibodies. A paper published in Nature of May 14, 2020 suggested that even though the RBD is less conserved, identical residues exist between SARS-CoV-2 and SARS-CoV RBD[3]. Further, these investigators reported that since RBD is the important region for receptor binding, antibodies that target the conserved epitopes in the RBD could also provide important clues for developing potent cross-reactive therapeutic agents against diverse coronavirus species, including SARS-CoV-2. The details of the virus replication are beyond the scope of this document; nevertheless much is covered in the immunological context later.

Transmission

Since the onset of the pandemic, several publications have appeared suggesting that the transmission of SARS-CoV-2 can occur by maintaining close contact with an infected person through his/her secretions such as saliva and respiratory droplets, which are expelled when that person coughs, sneezes, talks or sings. Respiratory droplets or aerosols that are <5µm in diameter from an infected person in close contact (within 1 metre) include virus that can reach the mouth, nose or eyes of a susceptible person and can result in infection. Indirect contact transmission involving contact of a susceptible host with a contaminated object or surface (fomite transmission) may also be possible. Respiratory droplet transmission can occur when a person is in close contact (within 1 metre) with an infected person who has respiratory symptoms (e.g. coughing or sneezing).

The virus affects all ages including the children as well as both sexes, but predominantly males (87% of those aged between 30-79, 10% aged <20 years, 3% of those above 80). Most investigators agree that it has a variable incubation period of 2-14 days with a mean of 5.2 days, mean time to symptoms of 5 days, mean time to symptoms of pneumonia of 9 days and mean time to death of 14 days.

The most plausible origin of the virus is from Bats, a mammal from where it spreads via human to human transmission through the shed droplets while talking, singing or sneezing and from the surfaces to human transmission because of the presence of the virus on surfaces for up to three days. It enters the human body through the mucous membrane of the eyes, nose or mouth.

The viral spike or the 'S' protein has a special affliction for the angiotensin converting enzyme 2 (ACE2 receptors). These receptors are found in several other organs of the body like the heart muscle, central nervous system, kidneys, blood vessels and the liver. Once the virus enters, it turns the cell into a factory, making millions and millions of copies of itself — which can then be breathed or coughed out to infect others.

The virus spreads rapidly in closed environmental conditions with no or poor ventilation and in crowded places with close physical contact. It is important to consider every surface and every person as a virus carrier.

Most apparently healthy people could actually be infected, but may remain asymptomatic carriers of the virus for months together. It is important to maintain a social distance of at least 2 meters from each other and wear face mask while meeting and greeting people outside of the home environment.

It is important to remember that the pandemics are fought on the ground, and not in the hospitals. The Mantra is to trace, track and treat by increasing the laboratory testing capacity several fold. From community mitigation to the individual containment, it is advisable to consider every surface and every healthy (asymptomatic) person as a virus carrier.

During the period of pandemic, the health authorities have the additional responsibility of maintaining a record of hospitalizations, improve general infrastructure and intensive care facilities in hospitals, and ensure bed availability for all those developing severe and critical stage symptoms. The primary goal is to save lives.

Monitoring deaths is important, especially when testing facilities are limited. Daily deaths are the best indicator of severity of the pandemic, although there is generally a 17 to 21 days lag period between the onset of infection and deaths. Countries with the best of health care facilities have reported <1% of deaths in symptomatic cases. Therefore total deaths multiplied by 100 gives a near expected number of symptomatic cases.

Other Coronaviruses

There are hundreds of coronaviruses, most of which circulate among such animals as pigs, camels, bats and cats. Sometimes these viruses jump to humans, often through an intermediary species and can cause disease. The first coronavirus was isolated in chicken embryos in 1937[4] causing a type of bronchitis in birds with potential to infect the poultry stocks. However, until the emergence of severe acute respiratory syndrome (SARS) in Guangdong, China in 2002/03, coronaviruses had been of greater concern only for the agriculture sector rather than public health.

First Coronavirus causing SARS

SARS-CoV of 2002 is reported to have a zoonotic origin and horseshoe Bats seem to be its natural reservoir. Palm civets and other as yet unknown animal species served as amplification hosts. Interestingly, shortly after a wildlife trade ban was imposed to control the SARS outbreak, there were no further naturally acquired human cases of SARS in Guangdong.

SARS-CoV is mainly transmitted among humans by the deposition of infected droplets or aerosols on the respiratory epithelium. In addition, transmission is infrequent during the first 5 days of illness, partly because of the low viral load in respiratory secretions during that phase. Excretion of SARS-CoV in sputa and stools may average 21–27 days after symptom onset, respectively.

Transmission of SARS-CoV among health-care workers and between patients in the hospital setting played a pivotal role in outbreak propagation. The virus was found to persist for up yo 2 days on environmental surfaces and 4 days in diarrheal stools. An estimated 8500 cases were reported in the outbreak, with fatality rate of at least 10%. At present, no circulation of SARS-CoV is registered.

Seven other Coronaviruses

Ever since the discovery of SARS-CoV, intense scientific efforts have been directed towards characterizing additional coronaviruses in humans and other animals.

Seven coronaviruses from two genera (alpha and beta coronaviruses) are known to infect humans, of which four are common and endemic – 229E, NL63 (both alpha), OC43 and HKU1 (both beta). These are known to cause mild upper respiratory tract infections in humans and gastroenteritis in animals (**Table 5**). People around the world commonly get infected with these four viruses. It is highly likely that there are additional unrecognized coronaviruses circulating in animals, and could infect humans through jump species in future.

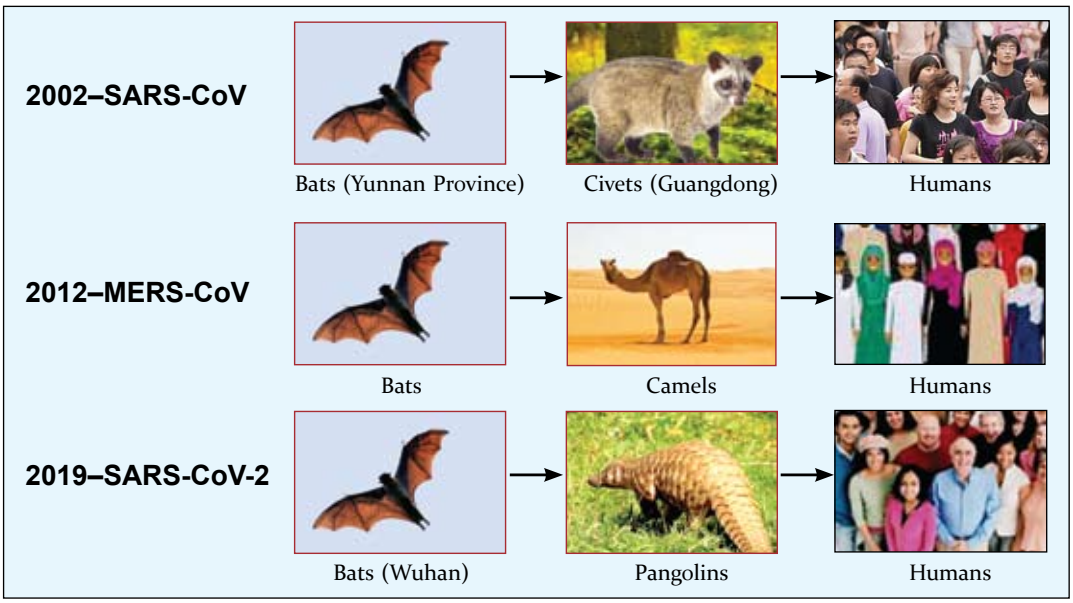
It is of interest to note that both alpha- and beta -CoVs are largely associated with mammals, whereas gamma- and delta-CoVs are largely harboured by avian species[5]. Since much of the genetic diversity of alpha- and beta-CoVs is associated with infections in bats, it has been suggested that bats are the main reservoir hosts for both alpha- and beta-CoVs.

The other three have emerged over the last two decades as highly pathogenic and pandemic human beta coronaviruses that cause severe pneumonia with acute respiratory distress syndrome, multi-organ failure and finally death. There is evidence to indicate that these have emerged from zoonotic events[6] (**Fig. 4**).

i. **SARS-CoV:** The first of these is the beta coronavirus (SARS-CoV) that emerged during an outbreak that emerged in China and 4 other countries and caused severe acute respiratory syndrome (SARS), identified at the end of February 2003. This is an airborne virus that gets transmitted through small droplets of saliva causing the first severe and highly transmissible new disease to emerge in 21st century. The typical incubation period is approximately 2-7 days which could extend as long as 10 days.

Table 5: Origin and clinical symptoms of human coronaviruses

HCoV	Incubation Period (days)	Clinical Symptoms	Year	Natural Host/ Intermediate Host
229E (alpha)	~2-5	Common cold-headache, sneezing, malaise and sore throat	1966	Bats /camelids
OC43 (beta)	~2-5	Common cold	1967	Rodents /bovines
NL63 (alpha)	~2-4	Moderate upper respiratory tract infection, severe lower respiratory tract infection, bronchiolitis	2004	Bats/ unidentified
HKU1 (beta)	~2-4	Common cold which can advance to pneumonia and bronchiolitis	2005	Rodents/ unidentified
SARS (beta)	~2-11	Fever, myalgia, headache, malaise, dry cough, dyspnoea, diarrhoea, respiratory distress	2003	Bats/ palm civets
MERS (beta)	~2-13	Fever, cough, chills, soar throat, myalgia, arthralgia, dyspnoea, pneumonia, diarrhoea and vomiting, acute renal impairment	2012	Bats/ dromedary camels
SARS-CoV2 (beta)	~3-6	Fever, dry cough, dyspnoea, myalgia, headache, loss of smell and taste, diarrhoea	2019	Bats/ pangolins?



- ✓ How do bats survive coronavirus as both can co-exist for long term?
- ✓ Do bats have a unique super immune system?
- ✓ Under stress, the balance between bats and the virus gets disrupted, causing the pathogen to multiply, mutate and transmit to humans

Fig. 4: *A view of the possible zoonotic transmission and evolution of the three most common coronaviruses seen at the beginning of the 21st century – SARS-CoV, MERS and SARS-CoV-2. (see text for details).*

Clinically, symptoms include high fever, sometimes with chills with other symptoms like headache, muscle pain and malaise. Some patients experienced diarrhoea during febrile stage. Severe cases require intubation or mechanical ventilation. The white blood cell count is commonly lower during early course of the disease and many patients presented with decreased platelet counts at disease peak. According to WHO, the case fatality of SARS was reported to be ~3%. Only supportive treatment is an option as no vaccine or curative treatment could yet be made available.

ii. **MERS-CoV:** The second was the MERS-CoV, again a beta coronavirus that caused the Middle East respiratory syndrome (MERS). Transmitted from an animal reservoir in camels, MERS was first identified in September 2012 in Saudi Arabia and it continued to cause sporadic and localized outbreaks. Approximately 80% of the cases were reported from Saudi Arabia alone followed by the United Arab Emirates and Republic of Korea. Finally, nearly 27 countries reported the presence of MERS infections.

Human to human transmission is responsible for most of the infections, while scientific evidence suggests that dromedary camels are the major reservoir host for MERS-CoV and zoonotic source of human infections. The mortality of this virus is ~35%, which is much higher than the others. It is possible that this higher mortality could be an overestimate due to missing of mild cases by current surveillance.

Typical symptoms included fever, cough and shortness of breath. Pneumonia and gastrointestinal symptoms (diarrhoea) were also commonly reported. Mechanical ventilation in intensive care units was required in cases with severe respiratory failure, which was more commonly observed in older people with weakened immunity and those with comorbidities like renal disease, cancer, diabetes and lung disease. Such high risk individuals were advised to avoid contact with camels or their products (milk, urine or meat etc.). Till now, treatment has only been supportive since no specific treatment or vaccine ever became available, although research to develop these was initiated.

iii. **SARS-CoV-2:** This is the third novel coronavirus to emerge in this century and it causes coronavirus disease 2019 (COVID-19). The disease first emerged from the Wuhan district of China in December 2019 and was soon declared a global pandemic by the World Health Organization on January 30, 2020. Most people infected with the COVID-19 virus experience mild to moderate respiratory illness and recover without requiring special treatment. However, people with advancing age, generally over 60 and those with underlying medical problems like cardiovascular disease, diabetes, chronic respiratory disease, and cancer are more likely to develop more serious illness.

The COVID-19 virus spreads primarily through droplets of saliva or discharge from the nose when an infected person coughs or sneezes. These droplets are too heavy to hang in the air, and quickly fall on floors and surfaces. The best way to prevent and slow down transmission is to be well informed about the causative virus and the process by which it spreads the disease. It is important to keep oneself as protected as possible through regular wearing of face mask, frequent and maintaining more than 2 meters of social distance while talking or interacting with others.

Unique Features of SARS-CoV-2

Incidentally, SARS-CoV-2 possesses unique characteristics of high infectivity, broad tissue tropism and generally causing severe pathology. It causes severe inflammation and damage to endothelial cells in the heart, kidneys, liver and intestines, suggestive of a vascular infection rather than a purely respiratory disease[7].

A noteworthy development has been the use of Cryo-EM for the first time to determine the structure of the SARS-CoV-2 spike (S) glycoprotein[8]. The study revealed

that the receptor binding domains (RBDs) of the virus tightly bind the free fatty acid like the Linoleic acid (LA). Such a binding linking LA and S directly presents a promising target for future development of small molecule inhibitors that could irreversibly lock S in the closed conformation and thus interfere with the receptor interaction. It may be mentioned that LA supplementation synergises with Remdesivir, a drug found to be effective for suppressing the virus replication in severe cases.

Generally speaking, the coronaviruses are relatively stable, although the same cannot be stated about the SARS-CoV-2. In the first and the largest analysis of molecular architecture of this virus involving sequence of 5,085 SARS-COV-2 strains causing two COVID-19 disease waves in the Houston area of United States, very important leads were obtained[9].

i. Virtually all strains in the second wave were found to have an Aspartic acid to Glycine replacement (D614G), a polymorphism that has been linked to increased transmission and infectivity.
ii. Such patients also had significantly higher viral loads in the nasopharynx on initial diagnosis. These results point to the strong possibility that the virus, as it has moved through the population, has become more transmissible, and that this may have implications for our ability to control it.
iii. The above information has been extremely helpful to understand the origin, evolution and trajectory of the possible future infection waves of this pandemic with potential effect of the host immunity in countering the same.
iv. Whether such virus variants arise due to a possible human host pressure is not clear, although the authors reported decreased reactivity of the neutralizing antibody with the D614G mutation.
v. As speculated, several regions of the world experienced second and even more waves of COVID-19 subsequently. India, on the other hand, escaped a classical second wave although smaller independent waves of infection appeared sporadically in various states and regions of the country.

Diagnostic Testing

The SARS-CoV2 suddenly appeared as a new virus with no proven therapy or a vaccine. Accordingly, diagnostic testing becomes an important tool for management of patients with COVID-19. The available laboratory test options include i) molecular test like the RT-PCR, ii) antigen test (point of care test, CARD test) and iii) antibody determination using automated platform and ELISA for IgG/IgM or total antibody i.e. IgG+IgM. The purpose of testing is diagnostic, sero-surveillance or to determine exposure and immunity levels. An estimated 40-50% of those positive for the RT-PCR test are asymptomatic.

The advantages of the RT-PCR test include speed and sensitivity, early detection and thus advisory on isolation, quick identification of infected persons which helps in the management and implementation of mitigation strategies in containment areas.

On the other hand, the disadvantages include the mandatory availability of BSL-3 or 2 level facilities, PPE training and properly trained and skilled personnel. There could also be false negatives due to improper sampling due to disease fear, sampling error, improper VTM (viral transport medium) and transportation errors.

Covid testing in India has been very regulated, as per the ICMR and government guidelines. RT-PCR with the sensitivity and specificity of >95% remains the gold standard investigation. Up until September-October 2020 when the infection numbers showed a steady rise, the Rapid Antigen test was permitted. However because of its poor sensitivity (<50%) and the increasing number of reported false negatives, the test reliability became an important factor.

The antibody tests are to be used only for the purpose of sero-surveillance and not for diagnosis. They provide good support for community screening of asymptomatic infections, contact tracing and for determination of levels of immunity at the community level. These could also be valuable for evaluating the results of vaccine trials.

As per ICMR revised guidelines, patients to be selected for testing include the symptomatic international travellers in the last 14 days, symptomatic contacts of laboratory confirmed cases, symptomatic healthcare workers, hospitalized SARI patients, asymptomatic direct and high risk contact of lab-confirmed cases, asymptomatic healthcare workers in contact with confirmed cases without adequate precaution and symptomatic ILI patients in hospital/clusters as identified by the Health ministry.

Pre-requisites before Testing

During a pandemic situation, it is mandatory to notify the result of each laboratory test done anywhere. To achieve that, a prescription from the treating physician along with the COVID-19 form of the ICMR giving patient details, history, clinical features, Government. ID is a mandatory requirement. Other pre-requisites include BSL-3 or at least BSL-2 facility, availability of trained personnel, efficient waste disposal system, judicious training of the staff and the use of PPE for sample collection and processing.

***Specimen type*:** nasopharyngeal/oropharyngeal swab. Experience indicates that nasopharyngeal swabs have better sensitivity. Accordingly, proper sample collection is a critical requirement of the whole process. Nylon swabs are generally preferred as coronavirus stays longer on synthetic material. Then immediately transfer to VTM (viral transport medium); shipped at 2-8°C with appropriate 3-layer packing. In later stages of infection, bronchoalveolar lavage or endotracheal aspirate provide a better sample source. Other studies indicated that saliva could also be better than nasopharyngeal or oropharyngeal swabs.

***RT PCR*:** Minimum two gene targets (E gene and RdRP gene) need to be pinpointed to declare a sample as RT-PCR positive. More the number of targets better is the sensitivity.

If the E gene, Rd RP gene and RP genes are positive, this confirms detection of SARS-CoV-2. If one gene is positive and the other is negative, the test is inconclusive and must be repeated with a fresh sample. If E gene and Rd Rp gene are both negative and only the RP gene is positive, the test is considered to be negative for SARS-CoV-2 virus.

It may be noted that the RT-PCR is a qualitative test as it does not give quantitative assay of viral load. The Ct (cycle threshold) value can give a clue about the severity of infection, but it is not a measure of the viral load. If the Ct value is low, this indicates high viral load. On the other hand, a higher Ct value is indicative of low viral load. Every laboratory doing the RT-PCT test for COVID-19 is expected to report the Ct value.

Rapid antigen test: Although the specificity of this test is fairly good, the sensitivity is only around 50-53%. On June 14, 2020, the ICMR recommended that for the rapid antigen test, time taken from sample collection to result reading should all be accomplished within one hour. For this, prescription/Form 44 are mandatory. If the antigen test is negative, but person has symptoms suggestive of COVID-19, then confirmation with RT-PCR test is mandatory.

Antibody tests: These are not used for diagnosis, but only for seroprevalence studies for community screening of asymptomatic infections and contact tracing. Notification of the test result is a must. However, prescription and form are not mandatory.

Two types of antibody test kits are available commercially to detect either both IgM and IgG or IgG alone. IgM appears first and then IgG. IgM appears around Day 4, rises to peak around Day 14 and disappears by Day 28. IgG appears around Day 8/9, rises to peak around Day 21 and then stays on. The longevity of IgG is reported to be between 4 to 6 months, after which time, it tends to wane.

Three types of seroconversions are seen in COVID-19. The most common is IgM seroconversion earlier than IgG. However, IgG and IgM may appear at the same time (synchronous seroconversion) and in rare cases, IgM seroconversion is observed later than IgG. The test results are interpreted as follows:

i. ***Total antibody (IgG + IgM) positive*:** This denotes confirmed exposure to SARS-CoV-2 with appearance of antibodies. If symptomatic, the patient must be referred for the more confirmatory RT-PCR test. If asymptomatic, then quarantine and repeat IgG is recommended after a gap of 7-10 days.

ii. ***Total antibody (IgG + IgM) negative:*** Exposure to the virus is not confirmed and therefore antibodies are not developed. However, if the person is symptomatic, it is recommended to do the RT-PCR test for confirmation. On the other hand, if asymptomatic, then this confirms a negative result.

Do Recurrent Mutations in SARS-CoV-2 Influence Virus Transmissibility?

SARS-CoV-2 is a positive single-stranded RNA virus that jumped species to the humans from an uncharacterized RNA reservoir towards the end of 2019. In order to adapt itself to the new host, the virus has gradually undergone several mutations leading to patterns of genetic diversity. The repository maintained by the Global Initiative on Sharing All Influenza Data (GISAID) has accumulated >70,000 complete high-quality genome assemblies as of September 21, 2020. Additional data are regularly fed into the repository from specific genome consortiums, including COVID-19 Genomics UK (https://www.cogconsortium.uk/data). The focus is to characterize the emerging mutations and classify their transmissibility potential.

Biologically speaking, mutations within RNA viruses including those in coronaviruses occur frequently because of the adaptation and survivability needs. In general, three processes have been identified: i) intrinsic mutations arise regularly as copying errors during viral replication. In the case of SARS-CoV-2, such a process may be infrequent due to the fact that coronavirus polymerases include a proof-reading mechanism ii) a possible recombination between two viral lineages co-infecting the same host leading to genomic variability of the virus. This is true of most viruses. iii) mutations can also be induced under pressure from natural host immunity involving RNA-editing systems[10].

The good old Natural Selection theory states that while a large majority of the mutations or genetic recombination are generally neutral, others may either be advantageous or deleterious to the virus. Mutations that are highly deleterious get deleted rapidly from the population, while those that are only slightly deleterious may be retained, albeit transiently. Consequently, the neutral and advantageous mutations are generally retained and these can reach high frequencies.

SARS-CoV-2 may not yet been adapted to the human host because of the limited association between the two so far. Van Dorp and colleagues[11] have recently analysed a data set of >46,700 SARS-CoV-2 samples obtained from 99 different countries across major continental regions. Using a phylogenetic index developed exclusively by them, they did not find even a single recurrent mutation in this virus that was convincingly associated with increased viral transmission. These investigators concluded that the genomic diversity of the global SARS-CoV-2 population is currently very limited, with little or no phenotypically divergent lineages.

This is an important finding that sets at rest all speculations on the variable potential of a COVID-19 vaccine across population groups. The recurrent mutations of the virus currently in circulation are primarily induced by the human immune system via RNA editing and these are evolutionary neutral.

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CHAPTER **2**

IMMUNOPATHOGENESIS IN COVID-19

Increasing evidence shows that immune patterns are closely associated with disease progression in patients infected with viruses. COVID-19 is a global health emergency of enormous magnitude influencing all aspects of human life. It is characterized by complex yet undefined mechanisms of immune mediated pathogenesis with remarkable heterogeneity and interplay at clinical, immunological and virological levels. Patients with this disease can develop pneumonia, severe symptoms of acute respiratory distress syndrome (ARDS) and multiple organ failure. Presence of comorbidities further complicates the recovery process due to generalized immune suppression.

Virus Entry into the Human Host

Despite widespread awareness on COVID-19, the path that the virus takes to enter the human body and cause tissue damage and pathology is not fully understood. SARS-CoV-2 enters the body through the nose, mouth or eyes and travels down to the alveoli in the lungs. The alveoli are tiny sacs of air where gaseous exchanges occur. Each sac of air or alveolus is surrounded on all sides by capillaries where red blood cells release carbon dioxide (CO_2) and pick up oxygen (O_2).

Two types of cells line the alveolar epithelium: Type 1 cells, also known as type 1 pneumocytes are extremely thin squamous cells that line most (95%) of the surface of the alveoli. These cells are not capable of cell division. They are thin enough so that the oxygen can pass right through. Type II cells, on the other hand are cuboidal in shape and are interspersed among type 1 cells. They secrete surfactant, a substance that lines the alveolus and prevents it from collapsing **(Fig. 1).**

The type II cells express the angiotensin converting enzyme-2 (ACE2) receptors, which are primarily utilized for viral entry through specific binding with viral spike proteins. The initial binding is facilitated by TMPRSS2 mediated activation and cleavage of viral S protein. This is followed by the injection of viral genomic RNA into the

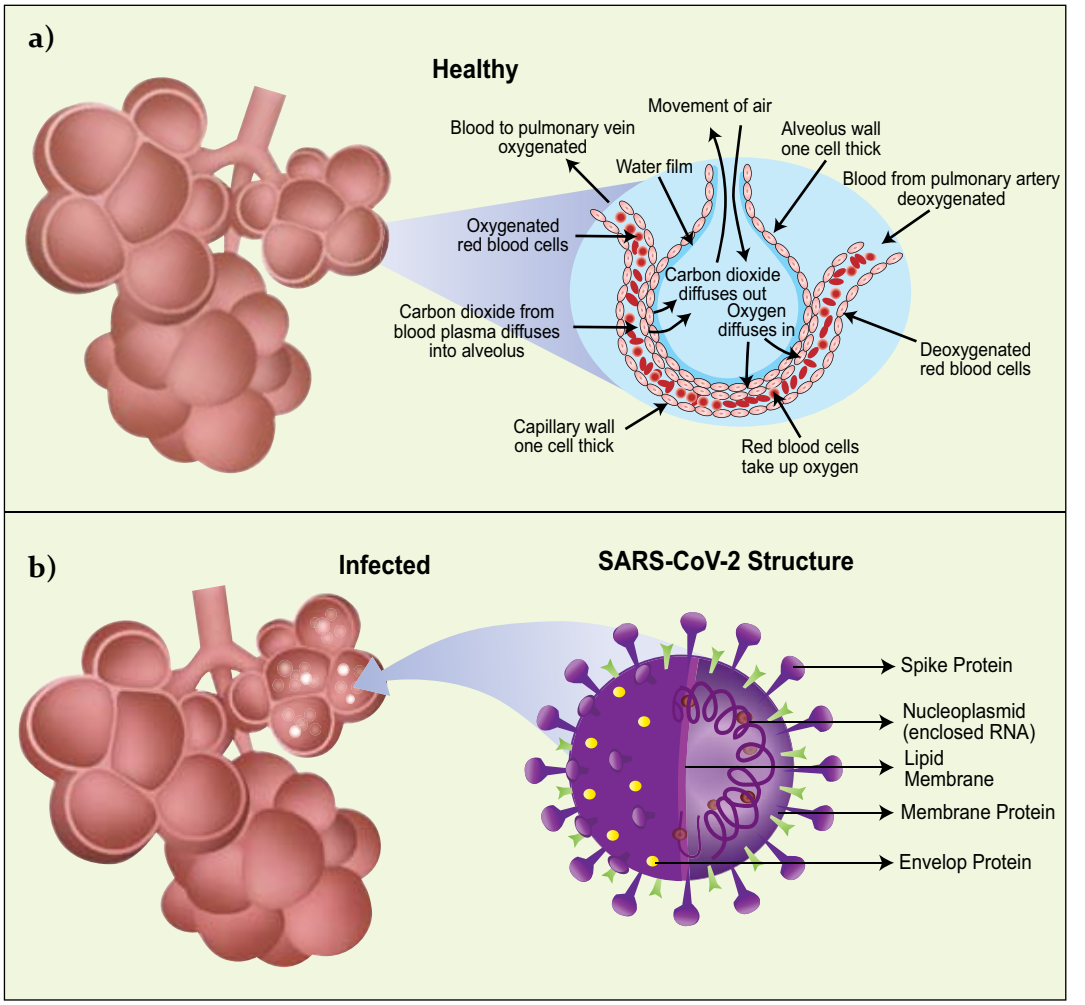

Fig. 1: *a) Diagrammatic sketch of an alveolus highlighting the process of gaseous exchange. The alveolus is surrounded by capillaries where red blood cells release carbon dioxide (CO_2) and pick up oxygen (O_2). Note the type I and type II cells lining the epithelium, b) alveolus from a SARS-CoV-2 infected person with hundreds of virions. SARS-CoV2 specifically infects the alveolar type II cells by binding to ACE2 receptors present on them.*

cytosol and viral replication. The process involves hijacking of the cellular machinery for viral genomic RNA synthesis, virion assembly and release of mature virions by exocytosis.

Viral Infection and COVID-19 Disease

The viral infection results in inflammatory signalling by the type II cells which recruit macrophages which in turn release cytokines leading to vasodilation and permitting more immune cells, particularly neutrophils to enter the site of infection from the

capillaries. These events cause the fluid accumulation and dilution of the surfactant inside the alveolus causing their collapse and decreased gas exchange.

Recruitment of neutrophils and their release of reactive oxygen species (ROS) kill infected cells. In severe cases, type I and type II cells get almost fully destroyed causing alveolar collapse and the resultant Acute Respiratory Distress Syndrome (ARDS). In case of further inflammation, the protein rich fluid can further enter the blood stream and cause systemic inflammatory response syndrome (SIRS), which may lead to septic shock and multi organ failure (**Fig. 2**).

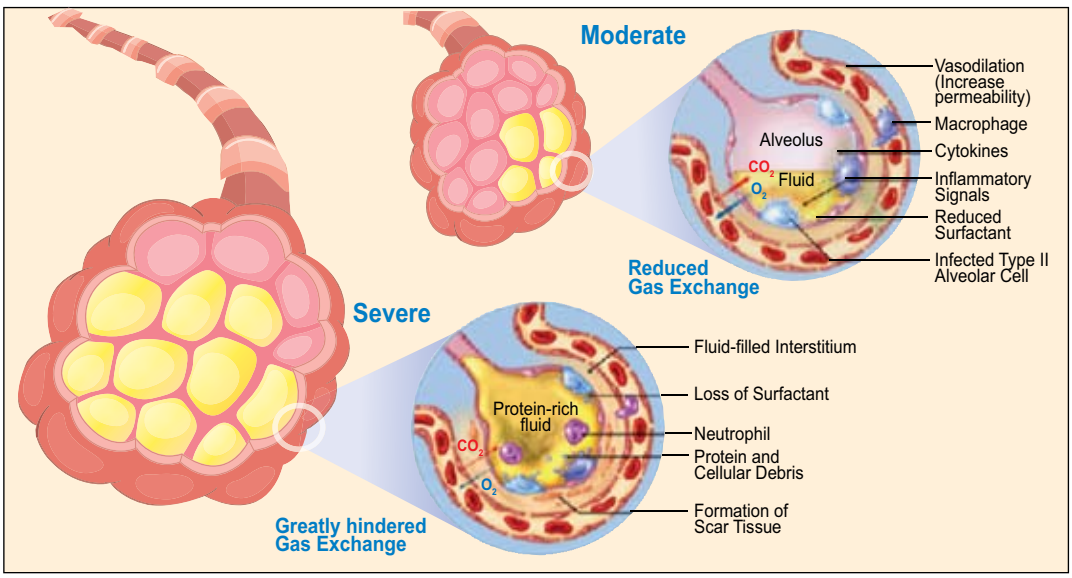

Fig. 2: *SARS-CoV2 infection mediated triggering of immune response leading to destruction of the healthy alveolar cells and compromise in gaseous exchange. a) patient with moderate disease. Note accumulation of fluid indicating inflammatory signal and collapse of alveolar cells due to reduced surfactants from type II cells, b) Patient with severe disease. The alveolus is filled with protein-rich fluid leading to greatly hindered gaseous exchange. In due course, neutrophils and other cells get accumulated. Eventually, the whole alveolus gets filled with the fluid indicating critical stage of the disease.*

Clinical Phenotypes and their Prognostic Kinetics

Clinically, COVID-19 varies in its manifestation that range from an asymptomatic state to variable symptomatic forms of mild, moderate and severe disease. Following infection with the contagion, there is usually a lag period of 4-5 days when the virus rather than travelling down the bronchus and into the lung parenchyma, stays in the naso-pharynx without causing any symptoms. The only clue about a possible infection could come from the travel history or details on close contact with another infected person in a crowded area. The infected person remains healthy and there is no laboratory test or a biomarker available to ascertain the possible disease course that this person could follow.

Majority of the COVID-19 cases (~80% or more) represent asymptomatic and/or mild category with almost zero mortality, if in quarantine and following adequate precautions and associated advisories **(Fig 3)**. At least 30% of all infected individuals are able to develop neutralizing antibodies against the virus, become immediately immune and stay healthy. Another 50% develop mild or low moderate symptoms lasting for 3-4 days, eventually becoming immune and recover with no mortality. All of these people are advised to stay in home quarantine.

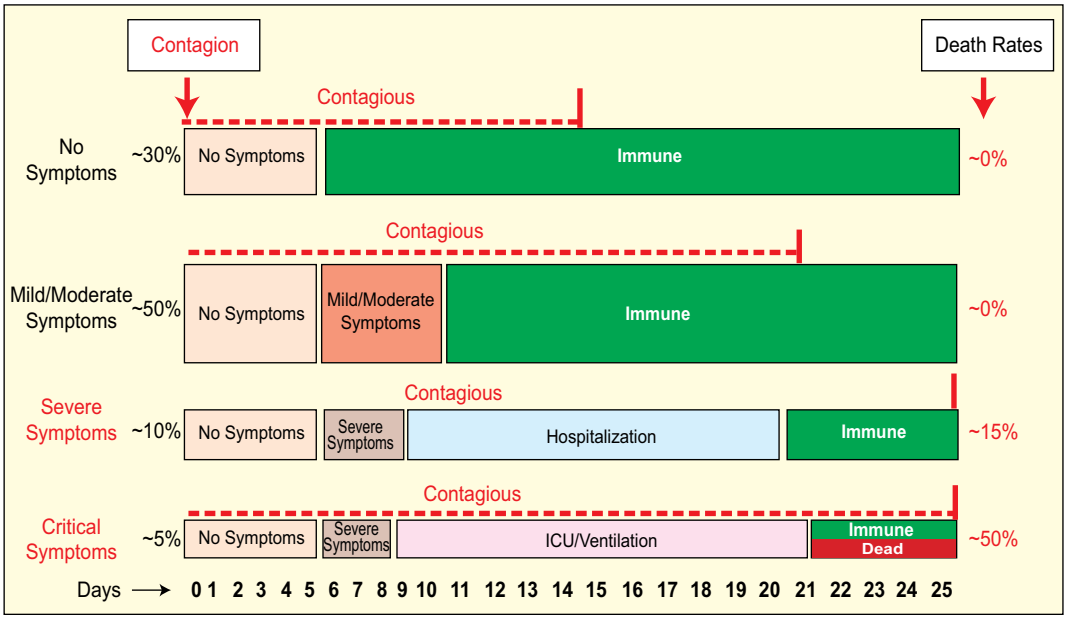

Fig. 3: *COVID-19 clinical phenotypes and their prognosis kinetics. See text for details.*

Only about 10% of the RT-PCR positive subjects develop moderate to severe symptoms requiring hospitalization and perhaps oxygen support. Most of them recover after a variable period of 10-15 days post appearance of symptoms, develop anti-COVID immunity and are discharged. However, approximately 15% of these patients gradually develop severe disease and may die.

A very small number (3-5%) of test positive patients, particularly those with additional comorbidities such as diabetes, cancers or other respiratory diseases etc. or the elderly people (>60 years) are specifically more vulnerable to progress towards severe disease. These develop very severe symptoms, require ventilatory support in the hospital intensive care unit and the death rate in this group is up to 50%. However, a good number among these can get cured and they develop high titer neutralizing antibodies.

In addition to dyspnoea, hypoxemia and acute respiratory distress, lymphopenia and cytokine release syndrome are clinically evident in severe COVID-19 patients

signifying immune homeostasis imbalance. This observed differential COVID-19 vulnerability signifies involvement of diverse spectrum of host immunity mediated through variable population specific genetic factors, microbial pressure, lifestyle, and the environment.

It is imperative to understand how the mechanisms of SARS-CoV-2 mediated immuno-pathogenesis could be harnessed translationally by developing preventive and therapeutic interventions. In the next two chapters, we attempt to review the influence of host immune response in influencing the clinical outcome and discuss immune correlates of COVID-19 for greater understanding of the disease process.

The Elderly and COVID-19 Vulnerability

Ageing mediated gradual deterioration of the immune system in elderly people i.e. immuno-senescence seems to be the most important factor involved in their COVID-19 vulnerability. The phenomenon is well correlated with various other infectious and autoimmune diseases as well as cancers in the elderly. Particularly, the presence of fewer naïve T-cells in older people is critical vulnerability associated factor, as these cells actually orchestrate the adaptive immune responses against pathogen that has never been encountered before.

The primary lymphoid organ "thymus", which is involved in T-cell education and maturation undergoes age related atrophy, with ~10 % drop in T-cell production by puberty as compared to childhood with consistent decrease thereafter. Incidentally, the dreadful situation of Italy (~10% COVID-19 mortality) could be directly attributed to the relatively higher median age of its population with many older people (>60 years). Neil Ferguson and his group published comprehensive results on March 30, 2020 strongly suggesting ageing related elevated risk of severity and deaths from the novel coronavirus SARS-CoV2[1]. They observed that clinical spectrum of COVID-19 disease shows a strong age gradient in risk of severity, hospitalization and death.

In this study, a substantially higher fatality ratio (>4.5%) was observed in older age groups (≥60 years), reached up to 13.4% in those aged 80 years as compared to those <60 years (1.4%). However, these results should not only be correlated with chronological age as the presence of underlying multiple chronic disease (comorbidity due to diabetes, cancers and cardiovascular diseases including hypertension) can make a younger individual even more vulnerable. Nevertheless, in general, the observed spectrum of COVID-19 disease shows a strong age gradient in risk of severity, hospitalization and death[2] suggesting population-ageing parameters as a correlate to disease outcome.

Apart from age, reports suggest that males are more vulnerable to COVID-19 in comparison to females which could be attributed to higher expression of ACE2 receptors in their lungs[3]. However, pregnant females may become more susceptible to COVID-19 due to fluctuating hormones affecting the ACE2 expression in different organs[4] and the altered immune responses at the maternal-foetal interface[5].

Besides the above factors, lifestyle is another critical factors as smoking, air pollution[6], high salt diet and low physical activity[7] have all been found to be linked to higher COVID-19 vulnerability, which could be due to dysregulating ACE2 and immune functions[8]. Thus the viral COVID-19 severity could possibly be predicted by the level of immune system imbalance and/or immune-senescence.

Conclusion: Overall, the observed clinical differences among the elderly as compared to the youth and children are attributed to their differential immune responses, as demonstrated by i) diminished ability of the elderly to mount a well-coordinated innate immune response. Increasing evidence shows that the elderly have compromised recognition of PAMPs (pathogen associated molecular patterns) by the innate immune cells resulting in stronger activation of PRRs (pattern recognition receptors), followed by an influx of immune cells and uncontrolled release of proinflammatory cytokines for compensation, ii) decreased ratio of naïve lymphocytes/memory cell ratio, iii) imbalance in the Th1/Th2 ratio along with decreased cytotoxic T-lymphocytic activity and CTL mediated immunity, iv) reduced presence of circulating plasma cells.

On the other hand in children, the SARS-CoV-2 infection gets resolved quickly due to the presence of less mature ACE2 receptors in them and rapid immune activation of immunocompetent cells. While in adults, negative immune regulation in the respiratory tract, increased ACE2 expression, ACE2 shedding and sACE2 production, all cumulatively can lead to uncontrolled immune response resulting in inflammation, cytokine storm and acute respiratory distress syndrome[9].

Imbalanced Host Immunity to SARS-CoV-2 Drives COVID-19 Disease

SARS-CoV2 infects host cells expressing the ACE2 (Angiotensin converting enzyme 2) receptor (expressed on alveolar epithelial cells) and TMPRSS2 through viral spike glycoprotein (S protein). This is followed by release of viral RNA genome and initiation of active replication as well as release of new virus particles. This initial interaction of S protein is an important target for viral neutralization and therefore being analysed primarily for defining various vaccines as well as therapeutic strategies[10].

In the cytoplasm, viral RNA is recognized by the cytosolic receptors that include melanoma differentiation associated gene 5 (MDA5), retinoic acid inducible gene 1 (RIG-1) and nucleotidyl transferase cyclic GMP-AMP synthase (cGAS). While in endosomes Toll like receptors (TLRs) recognize the viral RNA[9]. Release of virus leads to the pyroptosis of host cells which is a highly inflammatory process of programmed cell death commonly adopted by cytopathic viruses. This causes release of pathogen associated molecular patterns (PAMPs) such as viral RNA and damage associated molecular patterns (DAMPs) e.g. ATP, nucleic acids and ASC oligomers. The released DAMPs lead to hyper activation of cGAS-cGAMP-STING pathway and dysregulation

of IFN-I production. Thus, recognition of PAMPs and DAMPs through cognate pattern recognition receptors on neighbouring epithelial cells, endothelial cells and alveolar macrophages triggers the innate immune system leading to secretion of pro-inflammatory cytokines and chemokines viz. IL-6, IP-10, macrophage inflammatory protein 1 alpha (MIP-1alpha) and MCP-1 etc.

The resultant inflammatory milieu attracts monocytes, macrophages and T-cells to the local site of infection, augmenting further inflammation through IFN-gamma and activating a pro-inflammatory loop. This pulmonary recruitment results in lymphopenia and increased neutrophil-lymphocyte ratio as observed in around 80% of COVID-19 patients. Earlier studies have reported on the SARS-CoV evolved mechanism to evade innate immune machinery e.g. by lowering recognition by MDA5[11], dampening IFN-I production by a papain like protease and inhibition of STAT1 transport into nucleus during IFN signalling[12)].

Similarly, MERS CoV is also known to inhibit RIG-I induced type I and type III IFN production[13].

The antigen presenting cells such as dendritic cells engulf the released viral particles and process antigens for appropriate antigen presentation for activation of the adaptive immune responses. Antigen presentation is mediated by the human leukocyte antigen (HLA) system encoded by the most polymorphic gene dense region on chromosome 6 of man. It is a component of the major histocompatibility complex (MHC) that exists in almost all vertebrate species and its gene products are linked to infectious and autoimmune diseases. The HLA mediated antigen presentation results in initiation of both cellular and humoral immune responses. Please refer to chapter 4 for potential impact of HLA on viral replicative fitness at the population level.

For the sake of simplicity, viral immunopathogenesis can be explained through two contrasting immunological outcomes[14,15], namely a) dysregulated immune response and b) protective or calibrated immune response. In the case of former, an over accumulation of immune cells in lungs results in over secretion of pro-inflammatory cytokines which eventually damage the lung tissue **(Fig. 4)**.

Severe COVID-19 patients exhibited higher circulating levels of IL-2, IL-6, IL-7, IL-10, granulocyte colony stimulating factor (G-CSF), IP-10, MCP-1 MIP-1 alpha and TNF-alpha. The ensuing inflammatory milieu radiates to other organs causing multi organ damage with hepatic, renal and cardiac complications (liver, kidneys and heart). The antigen-antibody complexes can also mediate antibody dependent enhancement, thus further amplifying the magnitude of organ damage.

On the contrary, in a calibrated immune response, the initial innate immune activation and inflammation results in recruitment and amplification of appropriate virus specific T-cells through presentation of HLA restricted immuno-dominant conserved epitopes. These cells are able to eliminate the virus infected target cells and thus restrict viral spread. Further, T-helper cells provide help to the B cells to generate neutralizing antibodies. The latter are able to block viral replication followed

by phagocytosis of the neutralized viruses by alveolar macrophages, ensuring minimal damage, if at all to the lung tissue.

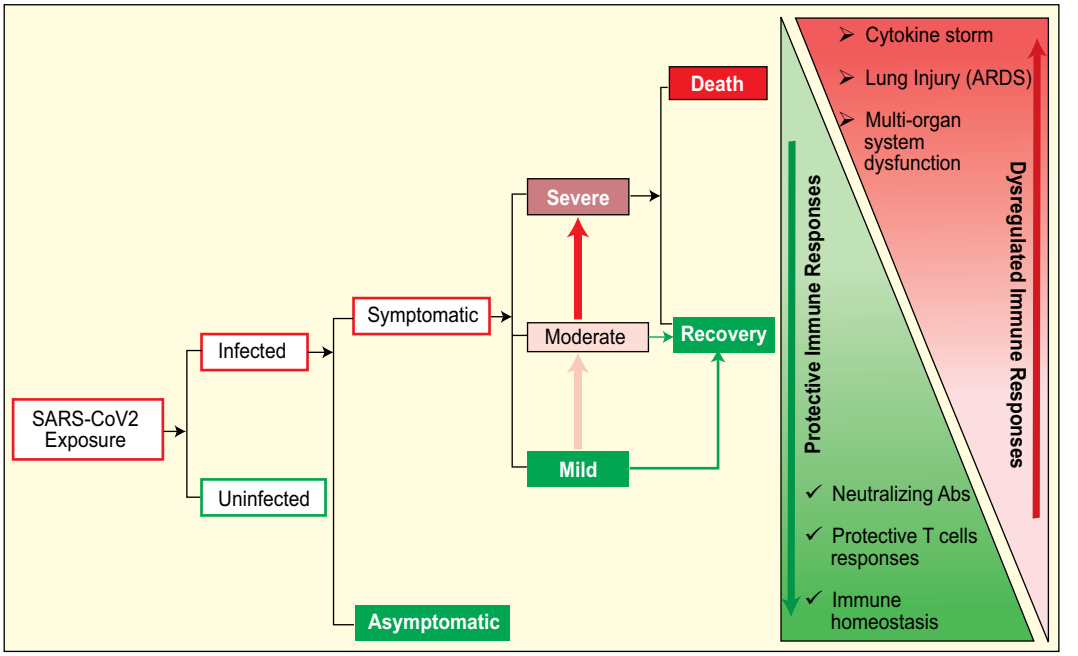

Fig. 4: *Clinical and Immunological Spectrum of COVID-19, reflected as a measure of the dysregulated or protective immune response following exposure to SARS-CoV-2. Patients with more severe disease are characterized with cytokine release syndrome and multi-organ failure.*

On the positive side, recruited cells in the lung efficiently clear viral infection resulting in recovery of majority of patients.

Haematological Impact of COVID-19

Ever since the beginning of the pandemic, questions have been raised on the possible haematological impact of COVId-19, particularly on leucocytes and platelets. A study published in the British journal of Hematology has suggested that the SARS-CoV-2 infection could indeed have a definitive impact on the red cell physiology, with an important role in oxidative stress[16]. The study is based on 50 hospitalized patients with COVID-19, of which at least 31 were in the ICU. None of the patients (36 males, 14 females) had a prior history of any hematological disorder.

Abnormal red cell morphology was found in all 50 patients, in particular, anisocytosis, spherocytes, stomatocytes and polychromasia. Strikingly, a few mushroom-shaped cells were also observed in 33 patients (66%) (mosaic, each image corresponding to a single patient). Their presence in two-thirds of patients with SARS-CoV-2 infection suggests a possible role for oxidative stress in the pathophysiology of the disease.

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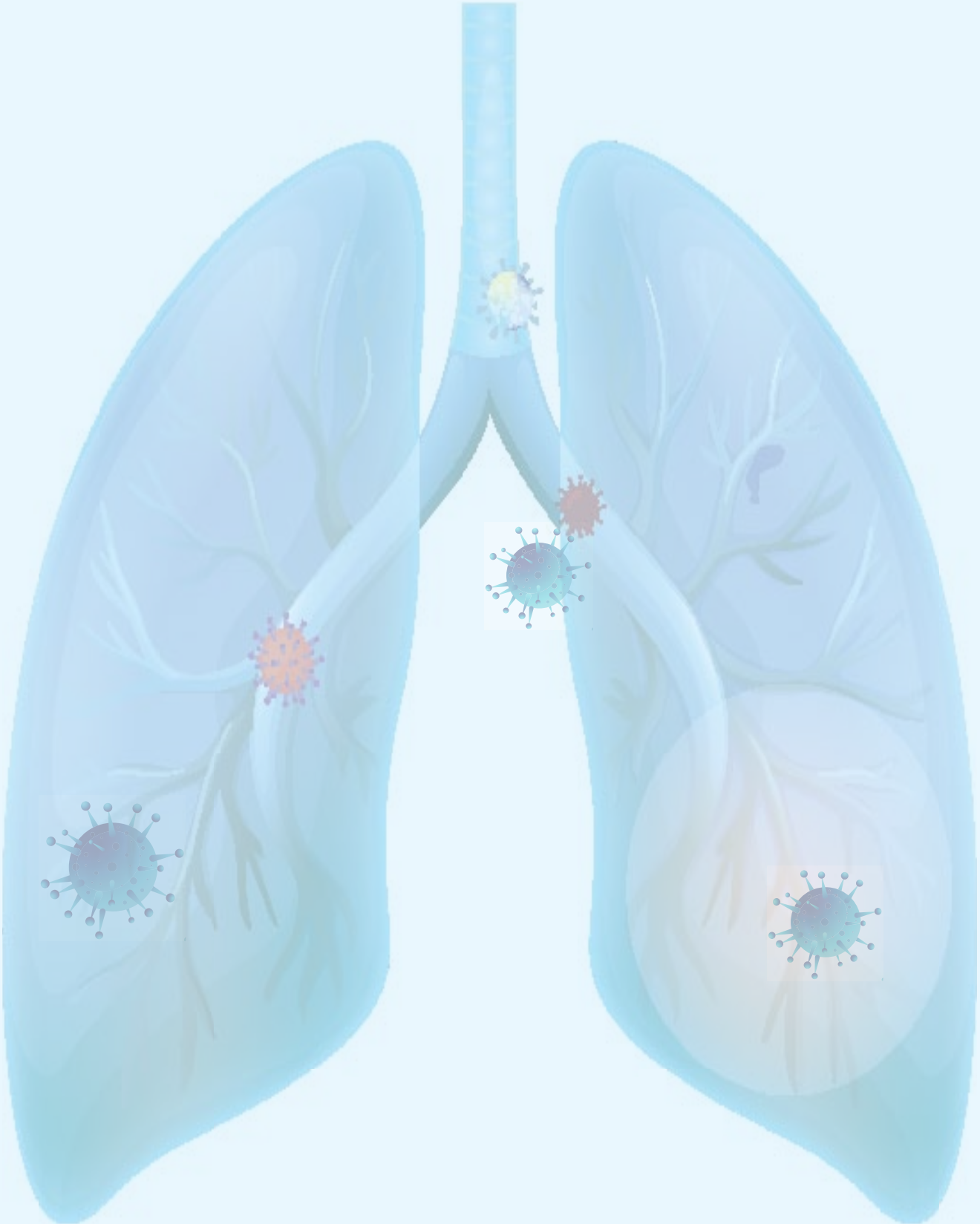

CHAPTER 3

IMMUNE CORRELATES OF COVID-19 VULNERABILITY

It is now clear that host immunity plays an important role as the most significant predictor of disease severity. In this context, the contribution made by innate and adaptive arms of immunity either individually or in tandem is critical in deciding the clinical course of COVID-19 infection. The former is pre-existing, and ready to attack, while the latter is stimulated by exposure to microbes and is always more potent. COVID-19 is a classic case of innate immunity hyperactivation and a case of adaptive immunity dysregulation and this imbalance drives the SARS-CoV-2 infection.

Innate and Adaptive Immunity: *two arms of the immune system*

Innate immunity, also called 'natural or native immunity' targets microbes and is a powerful early defence mechanism capable of controlling and eradicating infections even before adaptive immunity becomes active. Its components include epithelial barriers that help to prevent infections and cells like phagocytes, natural killer or NK cells and several plasma proteins including proteins of the complement system, all of which help to prevent infections (**Fig. 1**).

There is compelling evidence to suggest that a dysregulated innate immune response contributes to the clinical presentation of disease in persons with severe COVID-19 infection in which the tissue-resident macrophages initiate the process of epithelial damage leading to acute respiratory distress syndrome (ARDS). These macrophages are activated either by the damage-associated molecular patterns (DAMPS) such as intracellular contents released by the dying cells and/or proteins released during tissue injury or by the pathogen-associated molecular patterns (PAMPS) such as viral RNA or oxidized phospholipids.

Both DAMPS and PAMPS activate multiple innate immune pathways, through either the Toll-like receptors (TLRs), NLRP3/inflammasome activation or by triggering cytoplasmic DNA sensors.

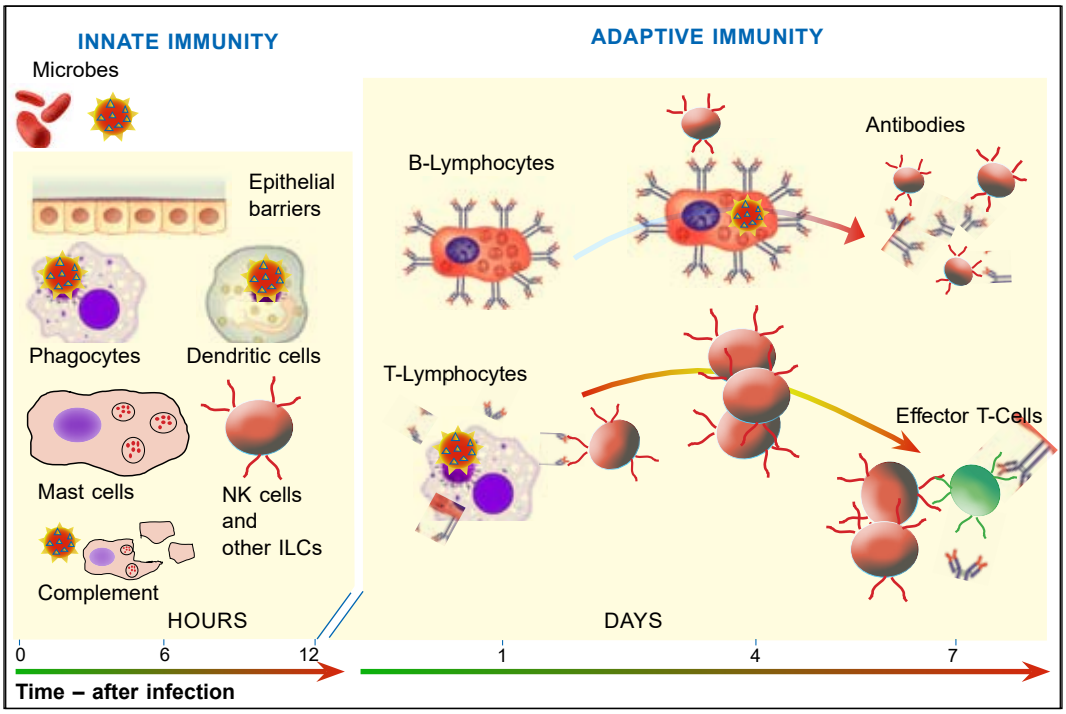

Fig. 1: *Specificity of innate and adaptive immunity. The former is always present and ready to attack. Many pathogenic microbes have evolved to resist innate immunity. Adaptive immunity is stimulated by exposure to microbes and is always specific and more potent, much needed. Note the cell types involved in the two arms of the immune system (Source: Abul Abbas).*

Adaptive immunity is designed to provide defence against extracellular and intracellular microbes leading to humoral and cell-mediated immune responses respectively. Classical teaching of immunology dictates that an intact HLA restricted T-cell mediated adaptive immune response is essential for clearing and maintaining long-term suppression of viral infections.

Extensive literature exists suggesting that patients with suppressed adaptive immune response have a significantly increased risk of viral activation. During acute infection, virally derived peptides activate both CD8+ and CD4+ T-cell proliferation and differentiation. Effective viral clearance that occurs within a week of the initial infection requires both CD8+ effector T-cell mediated killing of the virally infected cells as well as helper T-cell dependent enhancement of B-cells and thus antibody responses.

Chronic viral infections on the other hand, either evade or suppress adaptive immunity, leading to unopposed viral replication and a nonresponsive state, known as *'T-cell exhaustion'*. This feature is often accompanied by lymphopenia. Experience gained from numerous viral infections shows that viral dissemination is a key driver of severe disease.

Immunopathology of COVID-19

SARS-CoV-2 disrupts normal immune responses, leading to an impaired immune system and uncontrolled inflammatory responses in severe and critical patients with COVID-19. The most prominent of these is the presence of generalized lymphopenia, which is evident in such patients on hospital admission, suggesting it to be a significant predictor of severe disease[1]. The overall lymphocyte percentages show an almost 20% drop in severe cases. These patients also show marked reduction in CD4+ T, CD8+ T, and NK cell numbers as compared to the mild cases[2].

What could be the clinical implications of SARS-CoV-2 induced lymphopenia? One obvious outcome would be the higher vulnerability of such patients to microbial infections which could further promote disease progression and severity.

Interestingly, B-cell numbers were found to be mostly in the normal range[3] indicating that impaired B-cells are not as significantly impaired as T or NK cells. Besides lymphopenia, these patients exhibit lymphocyte activation and dysfunction (**Fig. 2**).

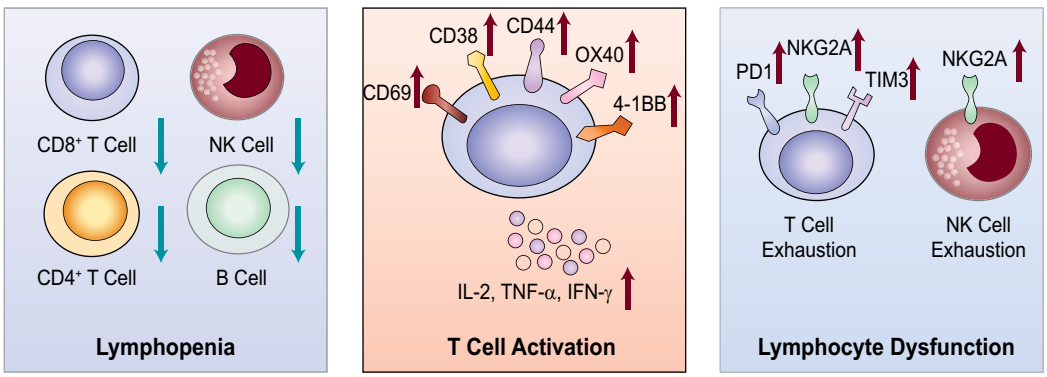

Fig 2: *Immunopathology of COVID-19 highlighting three hallmark events, namely lymphopenia, T-cell activation and lymphocyte dysfunction suggesting dysregulated immune response against the virus. Note the over expression of various activation and T-cell exhaustion markers and high levels of IFN-γ, TNF-α, and IL-2 released by virus specific T-cells. Details are provided in the text (modified from Yang et al)[3].*

A number of T-cell activation markers like CD69, CD38, CD44, and others have been evaluated and they all show an increased expression on both CD4+ and CD8+ T-cells of patients with COVID-19 as compared to healthy controls[4]. In this study, the virus-specific T-cells from severe cases presented with a central memory phenotype and high levels of interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and interleukin (IL)-2 compared with that of the mild group.

Simultaneously, T-cells in patients with COVID-19 show exhaustion phenotypes which is evident from reports suggesting an increase in programmed cell death protein-1, T-cell immunoglobulin domain and mucin domain-3 levels on $CD8^+$

T-cells in symptomatic patients, particularly those with severe symptoms. This was also the case with NK cells as seen by the increased expression of NKG2A on these cells (**Fig. 2**).

Abnormalities of granulocytes and monocytes were also consistently encountered in COVID-19 as shown diagrammatically in **Fig. 3**. Except neutrophils that showed a consistent increase in COVID-19, most other granulocytes were markedly decreased in most studies. Further, severe COVID-19 patients had increased cytokine production and this was the key characteristic of the severe disease. Data published by Diao et al[5] and others have shown that of the several cytokines evaluated, the three that showed most elevated levels in severe COVID were IL-1β, IL-6, and IL-10.

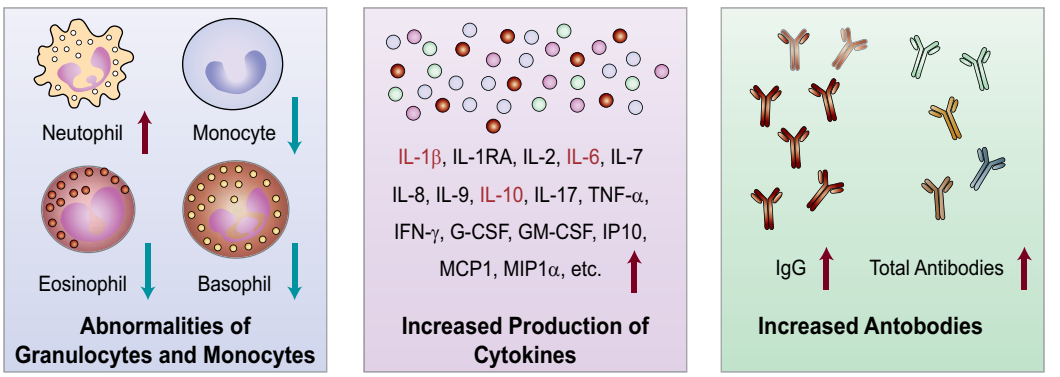

Fig. 3: *Immunopathology of COVID-19 highlighting abnormalities of granulocytes and monocytes, increased production of cytokines, especially IL-1β, IL-6, and IL-10 (shown in red arrows) and secretion of high titer antibodies, both total as well as IgG. Details are provided in the text (modified from Yang et al)*[3].

It is generally agreed that viral infections can induce severe shock syndrome and organ failure. This is true of both the influenza virus[6] as well as the Ebola virus disease[7]. Studies were conducted to see if a similar phenomenon happens in COVID-19, particularly in the wake of observed elevated cytokines. The data generated indicated that the 'cytokine storms' in COVID-19 could initiate inflammatory-induced multiple organ dysfunction, including lung injury that can lead to ARDS, respiratory failure, liver injury with alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamine transferase (γ-GT) upregulation. Further, such patients developed kidney injury with increased urea and creatinine levels, and heart injury with increased creatine kinase (CK) and lactate dehydrogenase (LDH) levels.

Thus elevated cytokine levels could be utilized as targets for developing immunotherapeutic strategies for halting severe syndromes. In non-severe or moderate COVID cases, most cytokines shown in **Fig. 3** were also elevated in the blood, although this increase was significantly lower than those in severe patients.

What about the B-cells and antibodies? Interestingly, patients with severe disease were characterized by an increased IgG response[3] and this observation could be used

as an important biomarker to discriminate between severe and mild cases. Indeed a high titre of total antibodies (IgM+IgG) and relatively high B-cell levels was associated with a worse clinical outcome. These results indicated that B-cell activation and proliferation in patients with COVID-19, especially in severe cases, is correlated with poor outcome.

Blockade of IL-6 Signaling

Of all the cytokines, IL-6 is most important in inducing the cytokine storm due to its ability to drive the over-reactive inflammatory response. Thus, targeting the IL-6/IL-6 receptor (IL-6R) signalling pathway is a promising strategy for relieving inflammation symptoms.

Tocilizumab, a humanized anti-IL-6 receptor antibody, has been developed and approved for the treatment of rheumatoid arthritis and juvenile idiopathic arthritis[8,9]. It has also been shown to be effective against cytokine release syndrome resulting from CAR-T-cell infusion against B-cell acute lymphoblastic leukemia.

The Chinese health authorities approved the administration of tocilizumab for blocking anti-human IL-6R for COVID-19 treatment as a clinical trial. The results revealed that while fever could get rapidly controlled, there was no dramatic improvement in disease severity. Similar trials were initiated with sarilumab, a fully-humanized monoclonal antibody that inhibits the IL-6 signalling pathway by binding to and blocking IL-6R. The results were not published.

Anti-COVID-19 Immune Response: *innate versus adaptive immunity*

During peak of the pandemic in April-May last year, Immunologists were the most active lot of researchers trying to explore combined contributions of the innate and adaptive arms of the immune system to both viral control as well as toxicity during COVID-19 infection. An excellent review article appeared in the journal of experimental medicine by the Memorial Sloan Kettering Cancer Centre, New York that put forward many facets of the anti-COVID host immunity[10]. The authors suggested that COVID-19 is a case of innate immune hyperactivation on one hand and of adaptive immune dysregulation on the other (**Fig. 4**).

***Innate immunity hyperactivation*:** Since cytokine storm is an important feature of COVID-19, the argument was put forward in favour of innate immune hyperactivity in driving the acute lung injury that defines severe COVID-19 infections. Earlier studies had indicated an important contribution of tissue-resident macrophages in the process of epithelial damage that initiates ARDS[11,12].

Macrophages are activated by either damage-associated molecular patterns (DAMPs) released from dying cells or pathogen-associated molecular patterns (PAMPs) such as viral RNA or oxidized phospholipids. Both DAMPs and PAMPs are likely generated during initial infection and through lysis of pneumocytes due to COVID-19 infection. These molecules activate multiple innate immune pathways,

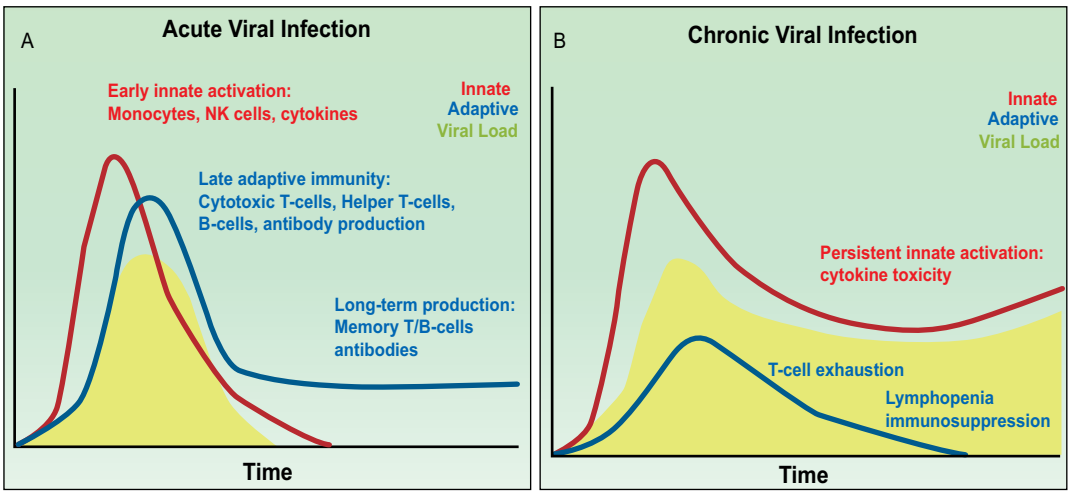

Fig. 4: *Immunity hallmarks of controlled and uncontrolled viral infections: a) during acute viral infections, early innate and adaptive immunity cause viral control and development of protective immunity. b) With passage of time, the uncontrolled viral infection leads to T-cell depletion, T-cell exhaustion and generalized immunosuppression.The innate arm of the immune system remains in a state of persistence and inflammation, while the adaptive arm shows a gradual decline (modified from Vardhana and Wolchok)*[10].

through either Toll-like receptors (TLRs) or NLRP3/inflammasome activation or other mechanisms.

The resultant signal transduction drives production of cytokines that aid in recruiting additional innate and adaptive immune cells at the infection site with distinct roles in antiviral immunity and tissue homeostasis. COVID-19 infected patients harbour an expanded population of circulating monocytes that secrete both IL-6 and IL-1β[13]. As a result, these patients often have elevated levels of serum IL-6, as well as lactate dehydrogenase as compared to the healthy controls. The latter is a marker of pyroptosis which is a form of non-programmed cell death driven primarily by inflammasome-mediated IL-1β production that results in release of cytoplasmic proteins and factors.

Elevations in innate immune cytokines leads to an innate immune mediated 'cytokine storm', similar to the cytokine release syndrome (CRS) observed in cancer patients receiving treatment with CAR-T-cells (chimeric antigen receptor – transduced T-cells), Such a cytokine storm is eventually responsible for the toxicity and end-organ damage mediated by COVID-19 infection[14]. Most patients with IL-6 elevation require mechanical ventilation and are ultimately associated with mortality.

Unlike the situation in CAR-T-cell therapy, clinical results of therapeutic blockade of circulating IL-6 with tocilizumab or siltuximab, the two monoclonals described above have not been very encouraging in COVID-19. The most plausible explanation for this has to do with macrophage activation, which occurs as a primary response to the viral infection[15].

Adaptive immune dysregulation: An intact T-cell mediated adaptive immune response is essential for clearing and maintaining long-term suppression of viral infections. Past experience suggests that patients whose adaptive immune system is suppressed, often end up with a significantly increased risk of viral reactivation.

Basic teaching of immunology dictates that during acute viral infections, virally derived peptides activate both naive $CD8^+$ and CD_4^+ T-cell proliferation and differentiation. Effective viral clearance, which occurs within a week of the initial infection, requires both $CD8^+$ effector T-cell mediated killing of virally infected cells as well as CD_4^+ T-cell dependent enhancement of $CD8^+$ and B-cell responses. However, T-cell dependent cytokine release and direct cellular cytotoxicity can also contribute to tissue inflammation and toxicity and accelerate mortality particularly during severe viral disease.

Following viral clearance, majority of the virus-specific T-cells undergo apoptosis. Nevertheless, retention of a virus-specific memory T-cell population is needed for long-term antiviral immunity (**Fig. 4a**).

Chronic viral infections either evade or suppress adaptive immunity for sustenance. Apart from failure to activate T-cell responses, these viral infections are characterized by persistent antigenic activation of T-cells, ultimately driving a state of non-responsiveness, which is known as T-cell "exhaustion"[16]. This phenotype has been described in numerous chronic viral infections and is often accompanied by lymphopenia.

COVID-19 is yet another example of a state of chronic infection. Since the clinical presentation of this infection is more consistent with a subacute rather than an acute viral illness, the question arises whether the clinical toxicity observed during severe disease represents a case of adaptive immune hyperactivity or suppression?

COVID-19 infected patients present to the hospital with a median incubation time of 5 days and are typically hospitalized for an additional 3–4 days before requiring either oxygen support or admission in an intensive care unit for mechanical ventilation. This subacute pattern of disease progression raises the possibility that immunosuppression, due both to T-cell depletion and exhaustion, contributes to COVID-19 viral persistence and mortality. The observed progressive and consistent lymphopenia in hospitalized patients is associated with their clinical deterioration. On the other hand, those with recovery of lymphocyte counts tend to proceed towards clinical recovery.

Based on the results of several studies, it is clear that a $CD8^+$ T-cell inflammation–driven lung pathology occurs early in COVID-19 infection, when there is a rapid expansion of short lived effector T-cells. Following this, persistent viral antigen load in the patient drives T-cell inactivation, exhaustion, and/or depletion.

Indeed, an active participation of the adaptive immune system in suppressing COVID-19 viral dissemination may also explain the association of COVID-19 disease severity with age. In Italy, for example several deaths that occurred in the initial phase of the pandemic was mostly of the very senior citizens above age 80 and those with

underlying co-morbidities. Age-related waning of adaptive immune function, also known as 'immunosenescence' is characterized by a loss of T-cell clonal diversity and a reduction in the capacity of naïve T-cells to undergo proliferation[17].

From the above, it is clear that a more restricted T-cell repertoire due to exhaustion as seen in COVID-19 and/or immune senescence as in the elderly is likely more prone to chronic viral infections. Conversely, the relatively diverse and expanded pool of naive T-cells may explain the relatively diminished severity of COVID-19 infections in children[18].

Important Literature on COVID-19 Specific Host Immunity

Since its emergence, the SARS-CoV-2 virus has been a subject of intense investigation to understand the cellular and humoral components of the immune response following infection of a susceptible host. The most significant predictors of disease severity relate to either activation or suppression of the immune response. We summarize here key findings from several studies on the role of innate and adaptive immune responses in contributing to the clinical course of COVID-19 infection, highlighting in particular the role of antibodies.

Innate Immunity

i. COVID-19 patients harbour an expanded population of circulating monocytes that secrete both IL-6 and IL-1β leading to elevated levels of serum IL-6 and lactate dehydrogenase (LDH) as compared to healthy controls. LDH is a marker of pyroptosis which is a form of nonprogrammed cell death driven primarily by inflammasome-mediated IL-1β production that results in release of cytoplasmic proteins and factors.

ii. Serum IL-6 elevation is reflective of the localized immune amplification through recruitment of additional immune mediators leading to innate immune-mediated 'cytokine storm' which is a characteristic feature of COVID-19 disease severity.

iii. In hospitalized patients, the level of IL-6 increase has been found to correlate with the need for mechanical ventilation and ultimately with mortality. The observed cytokine storm in these patients promotes neutrophil mobilization leading to COVID-associated lung toxicity characterized by alveolar flooding and fibrosis and finally causing Systemic Inflammatory Response Syndrome (SIRS). The latter may lead to septic shock and multi organ failure with fatal consequences. It draws parallel to the cytokine release syndrome (CRS) observed in cancer patients receiving treatment with chimeric antigen receptor-transduced T-cells (CAR-T).

iv. Therapeutic blockade of circulating IL-6 with Tocilizumab or Siltuximab has been attempted in COVID-19, but with mixed results. This is in contrast to

the similar attempts in CAR-T-mediated CRS where such a therapy has been more successful. Although the exact reasons for this observation are not clear, but may have to do with the process of macrophage activation observed in the two clinical situations.

v. For example in CAR T-cell mediated CRS, macrophage dependent production of IL-1 and IL-6 occurs secondary to T-cell mediated killing of tumour cells, while in COVID-19, it occurs as a primary response to viral infection. Hence rather than offering benefit, suppressing innate immunity in COVID-19 by IL-6 blocking monoclonal antibodies leads to further virus dissemination and consequently more serious disease.

vi. It is possible that a suppressed innate immunity is unable to enhance adaptive immune response against the infectious agent. As described below, interferon responses remain muted in severe COVID-19 infection due to the release of specific autoantibodies and this further leads to innate immune failure to control virus persistence.

Macrophages and DCs

i. These cells act as a link on innate and adaptive immune system. One of the hallmarks of symptomatic SARS-CoV2 is accumulation of activated pro inflammatory monocytes/macrophages in the lungs.

ii. Macrophages are the major players in inflammation associated COVID-19 through secretion of inflammatory cytokines. Both previously described coronaviruses and as well as SARS-CoV2 trigger NLRP3 inflammasome activation and secretion of proinflammatory mediators, for example IL-6, TNF-α, GM-CSF, IL-1β etc. resulting in cytokine storm[19].

iii. Patients with severe disease exhibit highly inflammatory monocyte derived FCN1+ macrophages in the broncho-alveolar lavage fluid, not observed in mild cases. Also, the CD14+CD16+ inflammatory monocytes in peripheral blood were found to be higher in severe cases as compared to mild ones. Further, infected macrophages expressing ACE2 can potentially migrate to blood and spleen leading to spread of the infection[20].

iv. Though the exact role of dendritic cells (DCs) in SARS-CoV2 infection is yet to be determined in details, cytokines and DAMPs can potentially activate and infect tissue DCs as observed earlier during SARS-CoV[21].

v. In SARS-CoV, active replication of virus in DCs is compromised resulting in release of inflammatory IFNs. After infection, DCs migrate to the draining lymph nodes to present viral antigens to T-cells, thus orchestrating the adaptive immune responses. It is known that coronaviruses modulate DC functionality and secretion of cytokines and chemokines[22].

Adaptive Immunity

i. Global attempts begun almost immediately to characterize the adaptive immune protective correlates during the COVID-19 outbreak. Initially, Zheng and co-workers[23] performed extensive immune analysis and observed elevated exhaustion levels with reduced functional diversity of T-cells linked to COVID-9 severity. This report suggested that the levels of IFN-γ and TNF-α in CD4+ T-cells were lower, whereas those of granzyme B and perforin in CD8+ T-cells were higher, particularly in the more severe patients as compared to those with mild disease.

ii. Cluster analysis in the above study revealed differential expression levels of both exhaustion (PD-1, CTLA-4 and TIGIT) and functional (IFN-γ, TNF-α and IL-2) molecules in clinically different patient groups. Severe patients had significantly decreased frequency of multi-functional CD4+ T-cells (+ve for at least 2 functional cytokines) as compared to those with mild disease.

iii. Another study reported on the key role of poly-functional CD4+T-cells in HIV-1 viral controllers. The authors presented evidence to suggest that a similar mechanism could have predisposed COVID-19 patients to severity[24]. However, a study of Li et al[25] reported a higher frequency of these potent cells in patients with severe SARS disease (caused by a most similar related virus) as compared to those with moderate disease.

iv. Another report[26] on COVID-19 patients revealed decreased numbers of NK and CD8 cells, but with an increased expression of NKG2A suggesting exhaustion. However, successful therapy resulted in restoration of these cell numbers and functionality with reduced NKG2A expression.

v. Similarly, another study observed decrease in total lymphocytes, CD4+ T-cells, CD8+ T-cells, B-cells and NK cells in COVID-19 patients to be correlated with clinical severity, while an increase in CD8+ T-cells and B-cells was seen following treatment[27]. These investigators put forward the view that CD8+ T-cells could act as independent predictor of disease severity and treatment efficacy.

vi. A report on a non-severe COVID-19 case by Thevarajan et al[28] analysed breadth of concomitant immune responses prior to patient recovery. The investigators observed increased antibody secreting cells (CD3-CD19+CD27hiCD38hi), follicular helper T-cells (CD4+CXCR5+ICOS+PD-1) and antibodies (IgM and IgG) that bound SARS-CoV2 in blood before symptomatic recovery.

vii. Another report by Huang et al[29] observed higher plasma levels of IL2, IL7, IL10, IP10, MCP1, MIP1-α, and TNF-α in critical COVID patients in the ICU.

viii. Similarly, Qin et al[30] suggested that the observed immune dysregulation in severe COVID-19 patients is characterized by lymphopenia (especially T-cells including Treg cells), higher leukocytes counts, neutrophil-lymphocyte ratio (NLR) and elevated inflammatory cytokines (TNF-α, IL-1, IL-6, IL-8). These

results highlighted the importance of surveillance of NLR and lymphocyte subsets in screening of critical illness and treatment.

ix. More recently, Hachim et al[31] attempted to explore the largely unknown landscape of antibody responses to SARS-CoV2. In this study, 15 different viral antigens were analysed and new targets of SARS-CoV2 specific immune response were identified. Among these, nucleocapsid, open reading frame (ORF)8 and ORF3b elicited the strongest antibody responses and identified COVID-19 samples at early and late time points of disease with >96% specificity. These results could potentially help in improving the serological assays further and lead to the development of second generation diagnostic tests.

x. Using IFN-γ based assays in response to peptides spanning SARS-CoV2, Peng et al[32] attempted evaluation of T-cell epitope landscape and observed significantly higher breadth and magnitude of T-cell responses in severe COVID cases as compared to those with mild disease. The identification of T-cell responses associated with milder disease could represent protective immune correlates and this information could be helpful in designing epitope based vaccination approaches.

xi. Further, Mathew et al[33] performed deep immune profiling of COVID-19 patients and revealed distinct immuno types with potential therapeutic implications. Their study on 125 COVID-19 patients analysed different subsets of B and T-cells. The data showed decrease in all populations of responding CD4 and CD8 T-cells (HLA-DR+CD38+, KI67+, or activated cTfh) between Day 0 and 7, while in contrast, HLA-DR+CD38+ CD8 T-cells as well as both KI67+ and HLA-DR+CD38+ CD4 T-cells were increased in severe patients.

xii. Recently, Rodda et al[34] observed that mild COVID-19 elicits persistent memory responses displaying hallmarks of antiviral immunity. Briefly, they analysed the multifaceted SARS-CoV2 specific immunological memory responses longitudinally in patients who recovered from mild COVID-19. The recovered individuals developed virus specific IgG antibody responses, memory B and T-cells which persisted for at least 3 months. The virus specific IgG memory B-cells increased over time. Further, the virus specific memory T-cells exhibited potent antiviral function as evidenced by secreted cytokines and expanded upon antigen re-encounter, whereas B-cells expressed receptors capable of neutralizing the virus.

xiii. Similarly, Ripperger et al[35] performed a serological study and observed that all cases including asymptomatic individuals seroconverted within 2 weeks after RT-PCR positive results. Neutralizing antibodies produced against the spike RBD and S2 remain stable for at least 5-7 months post SARS-CoV-2 infection.

xiv. More recently, Wauters et al[36] performed a single cell deep immune profiling of bronchoalveolar lavage (BAL) obtained from patients with mild and

critical COVID-19 and compared the same with BALs from non-COVID-19 pneumonia patients and normal lung. An efficient effector function and active (presumably antigen driven) expansion of CD8+ resident memory (Trm) and CD4+ T helper-17 (Th17) cells was observed in mild COVID-19, while in critical cases, these cells were more naïve. In critical cases, both CD4+ Th1 and CD8+ T-cells expressed exhaustion markers indicating evidence of inflammation linked stress.

It is clear that lymphocytopenia is the major hallmark of this disease. Patients who are able to recover this number by day 7-10 develop mild/moderate disease and eventually recover. However, those with persistent lymphopenia develop a more serious disease, often progressing to critical phase and eventually fatality. Some facts about COVID-19 immunity are summarized in **Box 1**.

Box 1: Immunopathology of COVID-19 immunity: important facts

- **There is an elevated inflammatory response:** but response to anti-inflammatory compounds is mixed.
- **Lymphocytopenia:** Unlike other infections, the lymphocyte numbers are decreased in COVID-19, especially in severe cases. This is similar to the situation in HIV infection and AIDS or in Hepatitis.
- **Lymphocyte recovery:** Patients who are able to recover this no. by day 7-10 develop mild/moderate disease and eventually recover. Those with consistent lymphopenia develop more severe disease, often go on to critical phase/fatality.
- **COVID-19 Immune Profile:** Besides lymphopenia, the immune profile of COVID-19 includes T cell activation, T-cell exhaustion and hypo-function, abnormalities of granulocytes (high neutrophils but decrease of eosinophils and basophils), decrease in numbers of monocytes, increased production of various cytokines in particular IL-1b, IL-6, IL-10. TNFa and others, and high titer of total antibodies. The dogma is that how does lymphopenia relate to T-cell activation as seen by high expression of cell surface markers like CD69, CD38 and CD44.
- **Cytokine Storm:** Increased production IL1b and IL-6 is indicative of an exaggerated immune response.
- **Plasmablasts** and antibodies are observed in peripheral blood in hospitalized patients, but they are still sick. Is it a paradigm? Does convalescent plasma have a role?
- **Is there a "typical" immune response in COVID-19?**

Interferons and COVID-19

Interferons (IFNs) are a family of signalling proteins that are released by the host cells as a result of infection with viruses. In a typical scenario, a virus-infected cell releases interferons causing nearby cells to heighten their anti-viral defences. Besides antiviral properties, these molecules play an important role in antitumor and immunomodulatory responses. Interferons act through autocrine and paracrine ways and involve activation of various immune cells, for example:

i. maturation of dendritic cells (DCs), their migration and antigen presentation,
ii. increase IFN-γ secretion and cytotoxicity of natural killer cells (NK),

iii. polarization of T-cells into Th1 and differentiation of B-cells into immunoglobulin secreting cells.

There are two major classes of IFNs: type 1 (IFN-α subtypes, IFN-β, etc.) and type II (IFN-γ) and these use distinct but similar receptor systems. Of these, the former first described in 1957, are ubiquitously expressed cytokines that activate intracellular antimicrobial systems and influence the development of innate and adaptive immune responses against viral infections. Their receptors activate an array of signalling pathways including induction of IFN stimulated genes (ISGs) via the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway. These regulatory mechanisms determine the biological outcomes of type 1 IFN responses and whether pathogens are cleared effectively or cause chronic infection or autoimmune disease.

Type 1 interferons are polypeptides that are secreted by infected cells and have three major functions

i. induce cell-intrinsic antimicrobial states in infected and neighbouring cells that limit the spread of infectious agents, particularly viral pathogens,
ii. they modulate innate immune responses in a balanced manner that promotes antigen presentation and natural killer cell functions while restraining pro-inflammatory pathways and cytokine production,
iii. they activate the adaptive immune system, thus promoting the development of high-affinity antigen specific T and B -cell responses and immunological memory.

The two most well defined type 1 interferons are IFN-α and IFN-β, while the former is produced primarily by the plasmacytoid dendritic cells, the latter is produced by several cell types. Their production is induced after the sensing of microbial products by the pattern-recognition receptors (PRRs) and by cytokines.

Type 1 interferons are protective in acute viral infections, but have either protective or deleterious role in bacterial infections and autoimmune diseases[37]**.** Neutralizing IgG autoantibodies against type I IFNs have been known to occur in patients with autoimmune poly-endocrinopathy syndrome type I (APS-1)[38] and in women with systemic lupus erythematosus[39].

The production of IFN-I during the very early phase of infection affects the disease course. For example, an early IFN-I administration protects mice model from lethal MERS-CoV infection as compared to delayed treatment resulting in fatal pneumonia and cytokine storm[40]. To this end, germline molecular defects in IFN cascade are linked to Mendelian susceptibility to severe viral infections[41]. Presence of autoantibodies against type 1 IFNs could prime the immune system towards redundancy following pathogen attack. Since these molecules help in modulating innate and adaptive immunity, their blockage by neutralizing auto-Abs could push the former in a state of hyperactivation and the latter in depressed mode, allowing the virus an uncontrolled growth. This is exactly what happens in COVID-19.

In a study published in Science on September 24, Paul Bastard and colleagues[42] have reported that over 10% of patients with COVID-19 pneumonia had neutralizing IgG autoantibodies against Type 1 interferons at the onset of critical disease. Interestingly, none of the 663 people in a control group with mild or asymptomatic SARS-CoV-2 infection had these damaging antibodies, while these occurred with a frequency of 0.33% (4 in 1,227) of the healthy general population. An interesting feature of this study was the preponderance of male patients (94%) with IgG auto-Abs against type 1 IFN with half of the gravely ill being over 65 years of age. This might explain the increased vulnerability of older people to severe COVID-19.

Preponderance of males with severe COVID-19 disease globally and with higher fatality rate is interesting. Women generally have higher rates of autoimmune disease; so one hypothesis could be genetic, due to X-linked recessive trait. Women with two X-chromosomes are protected, while men with one are not. Supporting this suspicion, one woman with a rare condition that silences one X-chromosome developed severe COVID-19 and she had autoantibodies (Jean-Laurent Casanova, Rockefeller University, USA).

More recently, Alexis et al[43] performed whole blood single cell analysis to analyse integrated contributions of neutrophils, monocytes, platelets, lymphocytes and serum. A coordinated pattern of interferon stimulating gene (ISG) expression was observed across every cell population in the mild COVID-19 patients, while this was nearly absent in patients with severe disease. Paradoxically, severe COVID-19 patients produced higher titers of anti-viral antibodies and lower viral loads as compared to those with mild disease course.

Antibodies to SARS-CoV-2

Several commercial assays have become available to examine the natural acquired immunity to COVID-19 by determining antibody levels to the virus at the population level. Sero-surveillance studies to determine the presence of virus recognizing antibodies have regularly been carried out both in India (Delhi, Mumbai and other big cities) and elsewhere in the most affected areas globally including Spain and Italy. An analysis of the combined results of these studies revealed the maximum antibody prevalence levels of ~20% or even less[44,45]. Clearly, the large degree of regional variation observed in seroprevalence and cumulative deaths is attributable to innate host resistance of the population under study.

The US which has recorded the greatest number of infections so far, there has been a shifting trend of seroprevalence of antibodies, with figures ranging from >20% in New York to below 10% in most states and throughout the study period[46]. In several other states, the sero prevalence stayed below 1%. These results indicate that despite the fact that the pandemic has now been around for nearly a year, most people do not present evidence of prior COVID-19 infection, as determined by testing antibodies to SARS-CoV-2.

The big question remains whether the detection of anti-SARS-CoV-2 antibodies by the laboratory-based approaches is a measure of the host protective immunity? It is possible that protection requires achieving a specific quantity of a particular subtype of antibody. Equally important is to assess the binding ability of these antibodies to the most critical immunogenic epitopes on the virus, which may differ from the epitopes that are targeted in the commercial assays. Thus, it is premature to state whether information on seroprevalence of antibodies to SARS- CoV-2 can actually translate into protective herd immunity as the virus continues to mutate.

Nevertheless, robust and well-designed seroprevalence trials at the population level have the ability to provide crucial information on the immunity levels against the virus, in particular if protective 'herd immunity' can be reached anytime soon. Population level herd immunity is much desirable to halt the continuing cycles of infection and disease. This aspect has been discussed in chapter 4.

Even though effective vaccine candidates have become available, infection control measures like the regular use of face masks outdoors, maintenance of adequate social distancing and frequent hand washing must be practiced regularly as the primary approach to manage the pandemic.

Vanishing Antibodies and Robust T-cells

As of February 3, 2021, the pandemic has caused over 104 million infections and >2.2 million deaths globally. Nevertheless, the risk of infections is not distributed uniformly across populations. The important question is if the development of antibodies in asymptomatic individuals and those who have recovered confer long-lasting protection from the virus. Do such people get reinfected, and if they do, will it be mild or a more severe disease? Most studies reveal that the SARS-CoV-2 specific antibodies tend to decline after a period of 4 to 6 months. However, this is neither unexpected nor alarming.

Do vanishing antibodies signify that the person doesn't have immunity anymore? Compelling evidence from studies conducted on other respiratory tract viruses indicates that host immunity can indeed last for several years. This was the case with the H1N1 virus that caused the 1918 influenza pandemic, in which adolescent survivors experienced protection from reinfection until several decades later.

For most infectious diseases, the waning of antibody titers is normal and does not necessarily indicate loss of protective long-term immunity. Immunoglobulin G tends to develop a week or 10 days post-infection and the titers rise by around two weeks as active plasma cells secrete antibody into the systemic circulation. Those then wane as the plasma cells actively secreting the antibodies senesce, whereas resting memory B and T lymphocytes continue to circulate for years to decades. Data from several studies suggests that memory lymphocytes mediate long-term immunity to infection even in the face of waning antibody titers.

Here it is important to mention about the contribution of cell-mediated immunity involving CD8+ cytotoxic T-cells. Their role in conferring protective immunity to SARS-CoV-2 infection has now become abundantly clear. The question is whether we can use T-cell responses as equal indicators of immunity?

An online paper from the Duke-NUS Medical School in Singapore published in Nature of August 20[47] makes two important points: One, in addition to neutralising antibodies, all of the recovered COVID-19 patients were found to have SARS-CoV-2-specific CD4 and CD8 T-cells that recognized multiple regions of the nucleocapsid (N) protein suggesting that these play an important role in this infection. Second, patients recovered 17 years after the 2003 outbreak still possessed long-lasting memory T-cells that displayed cross-immunity to SARS-CoV-2. The discovery is promising because other studies have also suggested that even though antibodies may fade within months, the all protective army of T-cells can stay in the circulation for years.

Lessons

SARS-CoV-1 and SARS-CoV-2 are closely related, with about 80% of their genome being identical. It is possible that much of the population in China and other SARS-CoV-1 affected areas still enjoy long-lasting immunity and thus have largely been spared of the current pandemic. Indeed memory T-cells induced by previous pathogens can shape susceptibility to and the clinical severity of subsequent infections.

Here it is important to mention that resistance to infection may accrue from previous exposure not only to seasonal endemic coronaviruses, but also other microbes, all of whom trigger both T-cell and antibody activity. For instance, the Indian population has been exposed to a vast variety of pathogens, leading to the generation of broad specific memory T-cells, ready to confer protection from new invaders. It will be interesting to screen the exposed, uninfected subjects to examine the presence of both neutralising antibodies and killer-T-cells that can protect against COVID-19 infection.

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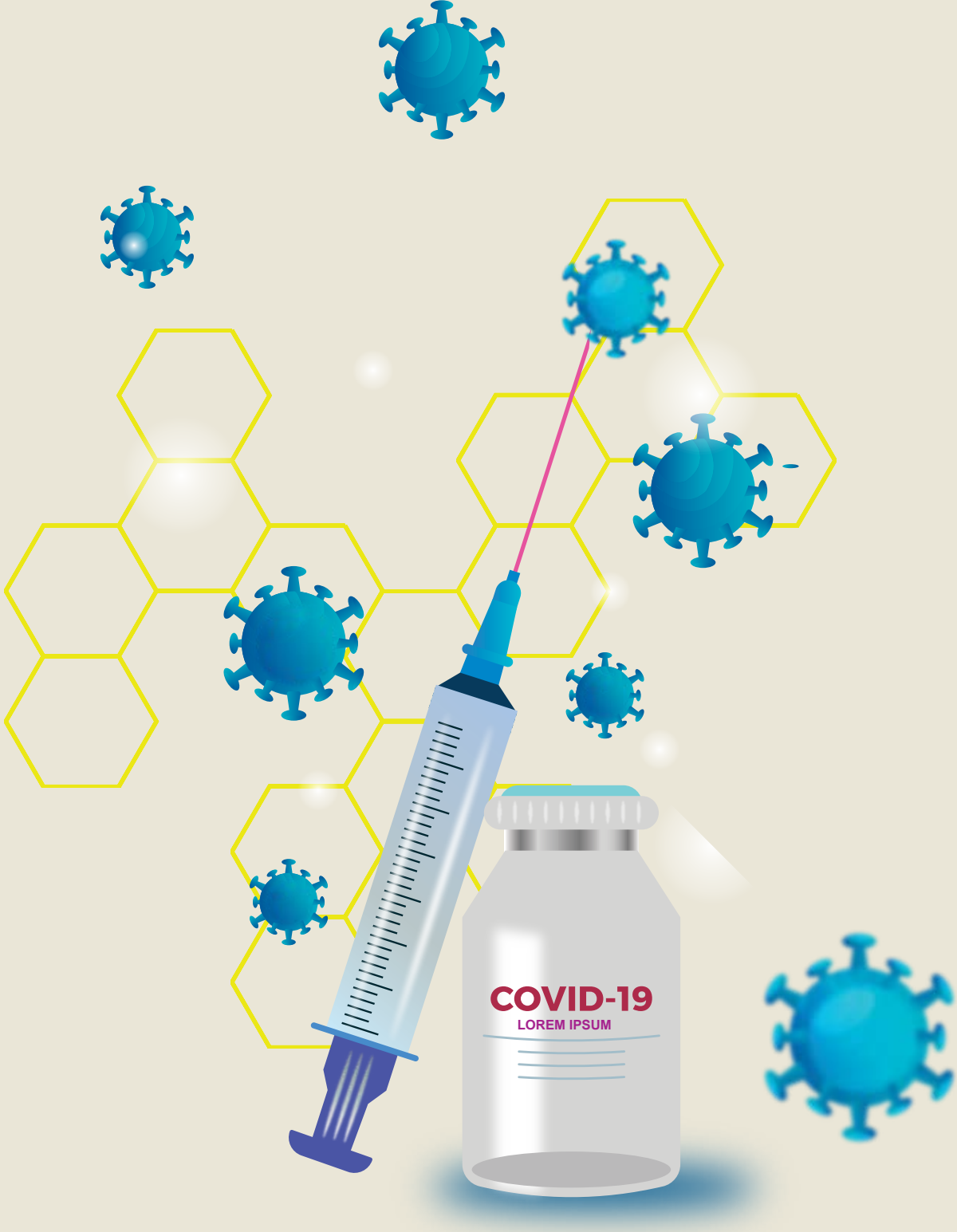
COVID-19
LOREM IPSUM

CHAPTER 4

IMMUNOLOGICAL CONSIDERATIONS FOR COVID-19 VACCINES

Long Term Immunity for COVID-19: *A conundrum for vaccine design*

Achieving long term COVID-19 specific immunity is the one of the major objectives of the ongoing vaccine strategies. Following viral infection, robust viral specific CD8+ cytotoxic T-cell, CD4+ memory T-cell as well as B-cell responses to resolve infection and their subsequent long-term immunological memory responses after recovery comprise the hallmark of an effective adaptive immune response. However, to date most vaccines rely on generation of effective and long-lasting memory B-cell mediated antibody responses and are less effective in eliciting CD8 T-cell responses. At least the data so far favors such a strategy.

During COVD-19 progression, immunoglobulin M (IgM) and immunoglobulin A (IgA) responses are initially triggered at ~ day 7 of the onset of symptoms. These responses start declining from ~ fourth week onwards, reaching negligible values by week 6 to 8. On the other hand, the most required viral specific immunoglobulin G (IgG) including the neutralizing ones begin to appear ~ day 10, attaining a peak at around 5-8 weeks before declining slowly ~3 months onwards. This observed short lived antibody response following the initial infection is due to the short lived B and plasma cells, a phenomenon seen also in other acute infections.

For all infectious diseases, the waning of antibody titers is normal and does not necessarily indicate a loss of protective long-term immunity. Actively secreting antibody forming plasma cells senesce over a period of time, whereas resting memory B and T-lymphocytes continue to circulate for years to decades. Existence of even a small effective pool of long-lived B-cells mediating immunological memory response through secretion of neutralizing antibodies is able to offer protection to future infections and is a positive sign for designing vaccines.

A recent report suggests that severe SARS-CoV2 infections compromise the germinal center (GC) response dampening production of long-lived antibody responses[1].

These GCs are transient microanatomical structures that form when the activated antigen specific B cells get help from follicular T helper cells (Tfh), where B cells undergo clonal expansion, affinity maturation and differentiate to memory B cells or long-lasting plasma cells.

Excessive TNF-α blunts the GC formation through blocking Tfh differentiation. Hence blocking TNF-α can potentially rescue GC formation based long-lived antibody responses along with reducing inflammation. Incidentally, short-lived humoral response caused by lack of germinal center has also been observed in SARS and MERS.

On the other hand, the virus specific helper and killer T-cells appear to attain peak within two weeks. Although robust data are still awaited, it is conceivable that these cells start to contract in 6-7 weeks possibly leaving imprints in the form of effective long-term immunological memory. Incidentally, Sekine et al[2] observed SARS-CoV2 specific memory T-cells in the majority of convalescent individuals including those with undetectable antibody responses as well as those with asymptomatic course of disease. These findings are encouraging and suggest that COVID-19 infection may result in long term immunity, but their role in protection from reinfection is yet to be explored.

Interestingly, analysis of samples taken before COVID-19 outbreak also revealed SARS-CoV2 specific memory T-cells suggesting the occurrence of cross-reactive T-cells in unexposed individuals. It is yet to be explored whether these T-cells generated due to cross reactivity against other coronaviruses in COVID-19 unexposed individuals will be protective or lead to disease severity[3].

Further optimism on the induction of long-term immunity following SARS-CoV-2 infection relies on reports of the presence of potent CD4+ and CD8+ T-cell memory responses elicited during asymptomatic infection even in the absence of an antibody response. Experts opine that achieving long-term COVID-19 immunity through natural infection alone may not be possible; nevertheless, recapitulating robust CD8+ T-cells and cytotoxic responses through vaccination is feasible, but is seemingly a challenging target.

Re-infections and COVID-19 Recovery: *a vaccine perspective*

SARS-CoV2 is known to elicit some level of immunity; however, predicting long-term protection from second/re-infection is of utmost importance for vaccine design and/or evaluating long-term vaccine efficacies. Incidentally, Deng et al[4] (2020) observed protection to reinfection in rhesus macaques supporting the prospects of an effective vaccine and long-term immunity. However, a few recently observed second/re-infections raise specific concerns and questions that relate to:

a) status and frequency of the second infections?

b) their symptomatic outcomes and disease severity status, and

c) possible implications for vaccines?

A recent report by Heidi Ledford in Nature has attempted these aspects[5]. The report specifically quoted two examples in this direction indicating exactly opposite clinical outcomes of re-infection/second infections. One of these relates to a man living in Hong Kong who got infected with coronavirus again after months of recovering from COVID-19 but didn't develop symptoms[6]. This is clearly suggestive of an effective memory immune response rescuing the host during subsequent infections, thus encouraging vaccine prospects.

On the contrary, another reinfection case from Nevada, USA resulted in more severe symptoms than the earlier infection suggesting failure of the immune system to protect against virus infection. More importantly, there was worsening of the clinical course of the disease, which could be explained only due to antibody mediated disease enhancement.

Incidentally, the viral genome sequencing of the above two cases of reinfections suggested that the first and subsequent infections in these two cases were through genomically different viral strains (separate variants of the virus) thus adding another dimension of complexity in COVID-19 immunopathogenesis and the need to stepping up sequencing facilities to evaluate multiple viral genomes in patients.

The few reports of reinfections or coinfections cannot provide conclusive evidence of long-term immune responses to SARS-CoV2. In this context, fresh outbreaks in some regions, more commonly known as 'second wave' of the pandemic need to be explored further for thorough evaluation of individuals re-exposed to virus (similar or different variant?).

However, in view of the overburdened testing facilities with fresh cases of the first wave, it is an obvious constraint to the tracking of second infections and/or re-infections. Overall, due to several limitations, evidence of re-infection has so far been limited.

Although long-lasting immunological memory is a hallmark of an effective and preventive vaccine strategy, the second/re-infections or disease enhancement raises the possible requirement of vaccine booster induced memory to reduce symptoms of the subsequent infection. But again, the reduced symptoms or asymptomatic nature during second/re-infection may render the unvaccinated and vulnerable population at higher risk.

Is Herd Immunity Achievable?

Herd immunity or community immunity is defined as a state achieved when a high percentage of a community becomes immune to a disease. More appropriately, it is an indirect protection from infection to susceptible (non-immune) individuals due to existence of sufficiently large proportion of immune individuals who compromise the disease spread across the community.

Since SARS-CoV-2 is transmitted through droplet infection from one human to another, non-availability of sufficient number of susceptible individuals could halt the

transmission. Because the infectivity of this virus is much higher than other similar coronaviruses, the threshold for most models estimate that the herd immunity in COVID-19 has to be at least 70%, if not more.

Conceivably, the herd immunity threshold is a point at which the proportion of susceptible individuals in a community falls below the threshold required for transmission. Of the known viruses, measles has been known to have communicability as high as COVID-19 and likewise, it also has higher requirement for herd immunity threshold.

Another important issue of concern is the immunological memory. While the measles vaccine is known to impart long term immunity, there is no such data as yet on SARS-CoV-2 infection. Most vaccines under trial are aiming at two vaccination doses rather than one and there is no clarity as yet on their long term effectivity levels. By the way, effectiveness assesses the vaccine's ability to do more good than harm when administered under standard conditions of healthcare delivery system.

Measure of Herd Immunity: *modelling studies*

Epidemiologically, herd immunity is considered to be achieved when an infected individual generates less than one secondary case on an average ($R_0<1$) where R_0 (Pronounced as R naught) represents the reproduction number. Briefly, the observed population specific heterogeneity in viral transmission can epidemiologically be explained in terms of R_0 which indicates an average number of secondary infections by an infected case in a susceptible population.

The R_0 of >1 suggests transmission potential to a number of individuals and spread of the disease (increase in value increases transmission exponentially), while R_0 of <1 is indicative of transmission to one or less than one individual resulting in disease eradication from the population (**Fig. 1**).

For COVID-19, the WHO initially anticipated the R_0 to range from 1.4 to 2.5. However, the real epidemiologic and/or transmission potential have been debatable in various reports. Recently, Liu et al[7] analysed results of 12 such studies (R_0 range, 1.5-6.68) and calculated the median (~2.8) and mean (~3) of estimated R_0 values therein, suggesting the estimated R_0 to be higher than that of SARS and MERS. These observed variations in different studies could be attributed to diverse methods used for modelling and the variables considered.

According to Hellewell et al[8], an R_0 of 1.5 requires successful contact tracing and isolation of <50% of contacts, while for R_0 of 2.5 and 3.5, >70% and >90% of contacts respectively will be required to be successfully traced and isolated, to contain the COVID19 outbreak. The control probability falls with lengthier delays from onset of symptoms to isolation and with increased asymptomatic transmission.

Briefly, to estimate the transmission potential and progression of the COVID-19 epidemic, R_0 may not be the only factor. This is because not much information is available so far on the mechanism of transmission through asymptomatic carriers.

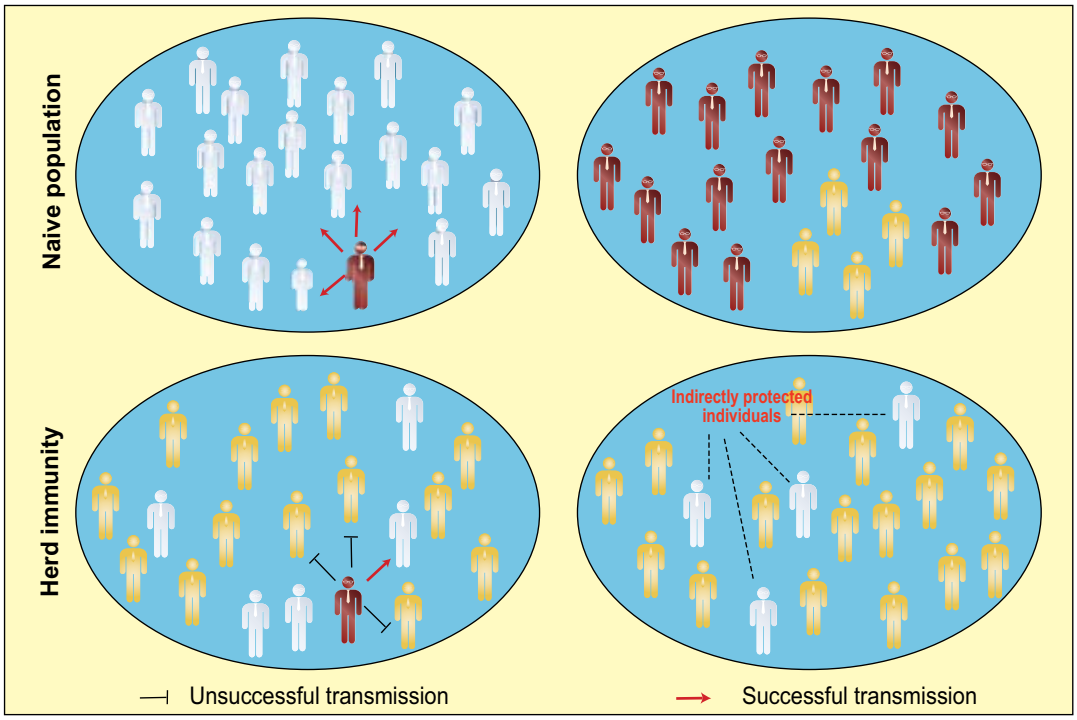

Fig. 1: *The principle of Herd Immunity.*

Moreover, it is important to improvise the sensitivity of diagnostic tests so that false negatives can be eliminated to a large extent.

How does it work?: Herd immunity can be achieved by letting the non-susceptible people, comprising mostly of the young without co-morbidities to develop resistance to COVID-19, either from being exposed to the virus or by vaccination. The former strategy was however, tried by Sweden without success. In fact, the country experienced several fold more number of deaths than their neighbours and the sero-prevalence figures remained very low to around 8%. Finally a lock down had to be imposed by the authorities.

The other extreme example is about Manaus, a Brazilian city of nearly 2 million residents near the Amazon forests which was devastated by a large outbreak of COVID-19 in June 2020. A large part of the population had got infected as was evident from the sero surveillance data suggesting ~66% positivity[9]. This large infection rate suggests that there were very few susceptible individuals left in the population for another outbreak. In fact, the fatality rate nosedived from around 120 per day to almost zero. *Nevertheless looking at the suffering of the population, achieving herd immunity through natural infection is indeed a bad idea.* The most critical question is how long does this immunity sustain? Despite the August slowdown of cases in Manaus, the numbers have started to rise again.

Although herd immunity can curb pandemics, it may not eradicate the disease. This is best illustrated by evaluating the historical precedent of the 1918 HINI influenza pandemic which was arguably more devastating than the current COVID-19 pandemic. After more than 2 years, over 500 million infections and ~50 million deaths globally, sufficient levels of population-based herd immunity must have developed, leading to cessation of the virus transmission and societal recovery.

Two methods: For achieving a state of herd immunity, basically there could be two ways:

a) through mass vaccination coverage which is exclusively dependent on developing an effective and safe vaccine (ongoing efforts covered in different sections)

b) as a result of effective 'immunological memory' due to prior natural infection. Although, this is theoretically conceivable, achieving herd immunity to COVID-19 through natural infection will remain a remote possibility due to the following factors:
   i) high transmissibility of the SARS-CoV-2 as compared to other known coronaviruses,
   ii) observed limited reports on re-infection of the virus in the same individual,
   iii) features of immunological correlates of memory make it a difficult proposition and
   iv) the observed lower susceptibility of children as compared to the adult population.

Although no data exist to define the exact threshold necessary to achieve herd immunity against COVID-19, modelling and extrapolation from similar diseases suggest that more than 70% of the population may need immunity for the viral replication rate to drop below 1. **(See Box 1).**

Box 1: Herd Immunity – Some Facts

1. **Generating immunity in younger people as a way of protecting the population as a whole:** The concept is based on the assumption that if >70% of all people (all <40 years with least mortality) get infected with SARS-CoV-2, it will be possible to control the epidemic and also prevent a 'catastrophic' second wave next year.
2. **Should we allow all <40 to get infected as a part of herd immunity plan?:** The answer is a definite no. The idea is purely hypothetical since deliberate exposure of the young and the vulnerable group would increase fatalities of the infected people by several fold.
3. **Could Vaccines generate 'herd' immunity?:** Yes, this is the only plausible solution. A more detailed explanation is given in the text.
4. **What else could be done to prevent a second wave?:** Intense surveillance, and strict observance of infection control practices (social distancing, face mask, frequent hand washing and a general change in life style) by all. Successful examples are South Korea, Taiwan, New Zealand, Hong Kong, Singapore and others.

Given the huge population size of India with over 130 crore population load, this will translate into at least 80 crore people getting open infected and becoming immune which is a very tall order. Further, the number of deaths could cross 10-12 million given the case fatality rate of ~1.4% which is inconceivable and unacceptable.

The only way to achieve a good level of herd immunity is through the availability of an effective vaccine and developing a carefully planned vaccination program. To this end, examples of measles, mumps, chickenpox and polio are encouraging as these infections (once very common) gradually transitioned to rare ones possibly through vaccine mediated herd immunity.

Reports suggest that protection against coronavirus species reinfection diminish with time (short lived immunity i.e. months to only few years not entire lives), thus achieving persistent herd immunity may only be attained through recurrent vaccination[10]. Incidentally, transmission dynamics modelling of SARS-CoV2 predicts that short term immunity (~10 months) would result in annual outbreaks while longer immunity (~2 years) would cause biennial outbreaks[11].

Though, the parameters of evaluating long-term immunity are beginning to be explored, mass serological surveys are absolutely needed to estimate and analyse the number of infected individuals in the population. It is also important to determine the number of those that are immune (antibody positive) so as to get an estimate of how far the community is away from reaching the herd immunity threshold, if at all.

Such attempts in countries like Spain and Italy, indicate 1-10% prevalence of antibodies, with peaks (10-15%) observed in heavily affected urban areas[12]. Further, in the absence of detectable humoral immunity, T-cell immune reactivity has been observed in patients' contacts[2] along with examples of cross-reactive T-cells in 20-50% of SARS-CoV2 naïve (unexposed) individuals[13].

Natural Immunity versus Vaccine Induced Immunity

There are reports in children and adults which suggest a lack of any sterilizing immunity as a result of previous coronavirus infection related possible cross reactivity[14]. Such emerging scientific updates on these parameters (long-term immunity and/or reinfections) are covered in other sections. Overall, the ~10 times higher mortality than flu warrants devastating consequences of achieving herd immunity through natural infection, thus rendering effective vaccine as the safest way to achieve herd immunity. Until the absence of such a vaccine approach, achieving herd immunity is far away as an advisable and/or achievable target.

It may be mentioned that the only disease to have been eradicated through vaccination and not by natural immunity is small pox. On the contrary, other respiratory tract viruses like measles, mumps, rubella and polio that once killed millions of people annually across the globe could induce long-term natural protective immunity against reinfection. Even for these viruses, vaccines alone could reduce the disease burden

by more than 99% (Data from US centres for disease prevention, *https://www.cdc.gov/vaccines/pubs/pinkbook/index.html*).

Are Indians more Immune to COVID-19?

A joint study done by the WHO and UNICEF found that a large proportion of the global population lack basic safe water, sanitation and hygienic conditions including the basic hand washing facilities. Most of these people reside in the developing nations and the observed higher incidence of communicable diseases in them is often attributed to their poor hygiene. Hence when COVID-19 struck, the biggest concern was that most of the low and low-middle income countries may have to face catastrophic consequences.

The surprise came from countries like India and may be those in South-East Asia and Africa. Although India stands at number two in terms of total infections, the case fatality rate in India has been the lowest at ~1.4%. Even more important is the very impressive recovery rate of >97% which is the highest in the world. In fact, the country's share of 'active cases' is the lowest at only 1.4% of the total infections (**Table 1**). France tops the list in terms of most active cases followed by UK and USA in that order.

Table 1: COVID-19 Dashboard: Percent recovery and active cases (country wise)
(Status: Feb 05, 2021https://www.worldometers.info/coronavirus/)

Country	Total Infected	Total Deaths	Recovery Number	Recovery (%)	Active Cases Number	Active Cases (%)
India	**1,08,03,533**	1,54,862	1,04,95,401	**97.1%**	1,53,270	**1.4%**
Turkey	25,08,988	26,467	23,96,199	95.5%	86,322	3.4%
Brazil	93,97,769	2,28,883	82,91,763	88.2%	8,77,123	9.3%
Russia	39,17,918	75,205	33,89,913	86.5%	4,52,800	11.6%
Italy	25,97,446	90,241	20,76,928	80.0%	4,30,277	16.6%
USA	2,72,73,889	4,66,989	1,70,31,625	62.4%	97,75,275	35.8%
UK	38,92,459	1,10,250	18,28,510	47.0%	19,53,699	50.2%
France	32,74,608	77,952	2,28,472	7.0%	29,68,184	90.6%

This raises the question if Indians are indeed more immune to COVID-19. Three factors seem to be playing a role in the observed higher recovery and very low active cases in India[15].

First, the broad-based immunity in the population due to the extensive microbial load from childhood onwards provides them with sturdier immunity, including to COVID-19. This one factor might have actually saved many lives from severe COVID-19. The Indian population has been exposed to a vast variety of pathogens, including bacteria, parasites and viruses leading to the generation of broad specific memory T-cells in the system, ready to attack additional foreign invaders. The three

main killers like Tuberculosis, HIV and Malaria have plagued India, Africa and several countries in the Southern hemisphere much more than the European and North American nations.

A recent study looked at the available data for 106 countries on parameters like demography, prevalence of communicable and non-communicable diseases, BCG vaccination status, sanitation parameters etc. Multivariate linear regression analysis revealed that while the incidence of communicable diseases correlated negatively while demography and improved hygiene, the higher incidence of autoimmune disorders correlated positively with COVID-19 mortality[16].

Since developing and underdeveloped countries generally experienced lower mortality rate due to COVID-19, this raises the possibility for an important contribution of the microbiome. A recent study analysed data from 122 countries, (80 high or upper middle income, 42 low or low middle income). Results showed a significant negative correlation between COVID-19 deaths /million population and the proportion of total population living in slums. The authors proposed that high microbial exposure particularly of gram negative bacteria could possibly induce type 1 interferon which might have a protective effect against COVID-19[17].

Second, epigenetic factors that include environment and food habits may also play a beneficial role for countries such as India. Much literature is already available in Ayurveda and other Indian systems of medicine on the definitive beneficial effects of Indian spices in augmenting immunity.

Third, and the most important is the possible influence of immune response or HLA genes whose major biological function is to present the invading foreign antigens to the immune system. As a consequence of the microbial load, the Indian population possesses a high genetic diversity of HLA[18], much more extensive than the Caucasian populations. Such genetic diversity of HLA could affect viral fitness and their genetic variants provide protection against such viruses.

Another source of indirect evidence comes from clinical COVID-19 studies which showed that rapid T-cell response appears to be crucial for recovery from COVID-19, while reduced functional diversity of T-cells in peripheral blood could predict progression of COVID-19.

In the context of CoV-2 coronavirus, the beneficial role of chloroquine and hydroxychloroquine has also been much talked about and debated, while there has already been an extensive usage of this drug at the community level in India – this too may ultimately prove beneficial.

Hygiene Hypothesis and COVID-19 Susceptibility

In the early 90s, the hygiene hypothesis was put forward to explain the rising incidence of autoimmune diseases and allergy occurring in the developed world[19,20]. Growing children in these countries were encouraged the extensive use of hand sanitizers and frequent hand washings. Further, they had frequent exposure to antibiotics for even

minor heath inconveniences. The hypothesis advocated that children exposed to certain environments such as farms, domestic pets and exposure to enteric parasites were less likely to develop allergies and some autoimmune diseases than those who experienced a more hygienic upbringing.

The unintended consequence of these hyper-hygienic practices have already proved counter productive since the children were not able to develop robust immunity due to a change in the balance of commensal microorganisms in their gastrointestinal tract, skin and other surface locations. In the present context of COVID-19, could the hygiene hypothesis also explain the observed higher susceptibility of Western nations (Italy, France, Spain, Germany, UK and USA) to severe consequences of COVID-19 infection?

It is conceivable that the hygiene hypothesis is a viable concept that applies to COVID-19 susceptibility. Coordinated research is needed, particularly by the epidemiologists and internists to determine if extensive exposure to multiple microbes in the environment, food and water, as commonly occurs among those that reside in depressed socioeconomic circumstances, may render them resistant to novel infections.

Lessons from Sero-surveys

Soon after the coronavirus pandemic struck in India in March last year, various sero-surveys have been conducted to determine the proportion of population that must have been exposed to the virus. Typically, such surveys use serology tests to check for the presence of antibodies among the population. If the tests detect sufficiently high quantities of a specific antibody in a person, it suggests that the person has been infected in the past.

Usually, these tests are conducted on a sample of the population which has been selected based on sampling techniques to ensure that the results can be scaled up to the entire population. The latest survey done in various parts of Delhi shows very interesting results. As compared to the earlier four surveys, the one completed recently in January 2021, involving >28,000 individuals showed that over 56% of the population may have already been exposed to SARS-CoV-2 (**Table 2**).

Table 2: Results of the five sero-surveillance done in Delhi area

Round	Period	Sample Size	Test Employed	Overall Exposure (%)
1	June-July, 2020	21,387	ELISA	23.4%
2	August, 2020	~20,000	ELISA	29.1%
3	September, 2020	~20,000	ELISA	25.1%
4	October, 2020	15,015	ELISA	25.5%
5	January, 2021	28,000	CLIA	56.1%*

Highest: South East Delhi 62.2%, Lowest: North Delhi: 49%
ELISA = Enzyme linked immunosorbent assay, CLIA = Chemiluminescence enzyme immunoassay
Note: National sero survey done by the ICMR in Jan 2021 shows ~21% positivity

The reasons for the very impressive results of 5th survey are not fully clear. Several factors could be in play including use of better testing technique of chemiluminescence permitting evaluation of both recent and past infections, higher number of infections experienced in August-September, 2020 and the resultant recovery from them, and truly declining infection rates in several regions of India due to impending herd immunity.

Similar results have been reported from other big cities like Pune and Mumbai reporting >50% natural immunity. This is in contrast to the national survey completed by the ICMR in January 2021, reporting a figure of 21%. The ICMR survey cautions that more than 3/4th of the population is still susceptible to the virus. The general public is advised to maintain COVID appropriate behaviour all the times.

It may be mentioned that high antibody positivity should not be taken as a signal for the advent of herd immunity in big cities. While it is definitely a positive sign, robust and more homogeneous herd immunity can be achieved only with vaccines and not through natural infection.

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CHAPTER 5

GENES MAY DECIDE THE CORONAVIRUS DISEASE COURSE

As per the COVID-19 organogram, there is a considerable disparity both in infection numbers and death rates among nations with the highest rates of fatalities recorded in North America, Western Europe and countries in South America like Brazil and Mexico, and the least in Africa and the Indian sub-continent. India with its huge population and several densely populated pockets has done exceptionally well by keeping the CFR as low as 1.4 to 1.54% throughout the pandemic. What is even more remarkable is the observed heterogeneity in disease presentation with most infected people remaining asymptomatic or presenting with mild symptoms, whereas among those who require hospitalization, only a small percentage develop critical symptoms and lose the battle. As per the Ministry of Health statistics, age and underlying co-morbidities account for much of this disparity.

Why some people with coronavirus have no symptoms and others get extremely ill is one of the pandemic's biggest puzzles. Genetics could help to explain why most people infected by the virus never get sick, while others may develop serious symptoms leading to death. In other words, a person's DNA profile could determine the course of COVID-19 disease.

In the classical approach to define the possible role of genetics, experts employ the highly sophisticated techniques of Molecular Biology to scan the DNA of a set of patients and an equal number of healthy controls (usually upwards of a thousand each), and look for any informative markers from among millions of DNA sequences and ascertain if any of these confer susceptibility and/or protection from that disease. Comprehensive efforts are ongoing to systematically utilize the enormous magnitude of data on genetic sequences. Further, specific bioinformatic tools and databases are now available. For example, archival data from a plethora of genome wide association studies (GWAS) on a variety of human diseases can be accessed from the NCBI

Database of Genotype and Phenotype (dbGaP) located at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gap.

In infectious diseases like leprosy and tuberculosis, both candidate gene and genome-wide association studies (GWAS) have been applied and these have provided significant insights into their genetic basis[1-4]. These studies identified informative genetic loci and allelic polymorphisms that determine, in part, genetic susceptibility to such infections. Similar studies of viral infections like SARS-CoV-2 are critical not only for understanding the disease pathophysiology, but also elucidating why COVID-19 manifests differently among individuals, and thus help in designing more effective vaccines and/or antiviral therapeutics. Such studies can also help pinpoint if emerging viral strains are linked to more severe clinical outcomes or if individuals harbouring certain alleles are differentially susceptible to disease.

A seminal example is the loss-of-function mutation reported in HIV whereby a deletion in the CCR5 gene, known as Delta 32, expressed as *CCR5Δ32*, abrogates expression of CCR5 on the host cell surface and renders homozygous individuals resistant to the infection[5,6]. Identification of such associations for SARS-CoV-2 could help prioritize individuals for treatment once an effective vaccine or antiviral therapy is developed.

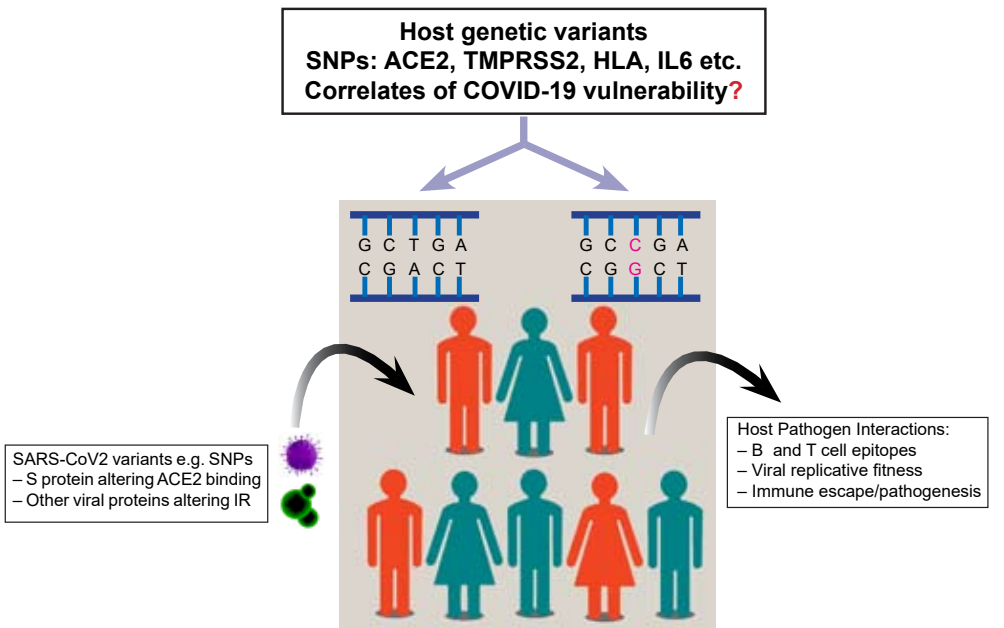

Fig. 1: *Host and viral genetic variations can modify the immunopathogenesis of COVID-19 and host immune response. Viral genomic variations in S protein can alter the viral binding efficiency to ACE 2 receptors. Similarly, other non-structural viral proteins can alter the immune response against viral target antigens. On the other hand, host genetic variations in other genes including ACE2, TMPRSS2, HLA, IL-6 etc. could differentially define the COVID-19 vulnerability in a population specific manner.*

How the host and viral genomic alterations could potentially influence disease pathogenesis and host immune response and thereby vulnerability to COVID-19 is summarized in **Fig. 1.** Single nucleotide-polymorphisms (SNPs) in viral S protein affect the viral binding efficiency through modified interactions with ACE 2 receptors. Similarly, alterations of viral non-structural proteins compromise immune response raised as a result of vaccine or natural immune response. Further, host genomic variations in ACE2, TMPRSS2, HLA, IL-6 etc. could affect population specific immunity and vulnerability to COVID-19 differentially.

From the above, it is evident that genetic variability both at the host as well as the pathogen level could affect the disease outcome. It may result in compromised viral replicative fitness due to host's immune pressure on one side and immune escape to B and T-cell epitopes on the other side.

Identifying genetic variants associated with the immune response and clinical outcome of coronaviruses could provide very useful information. Some data on these lines are already available for SARS-CoV-1, although the literature on SARS-CoV-2 is still preliminary. Large consortium approaches like COVID-19 Host Genetics Initiative, COVID Human Genetics Effort, HLA and Immunogenetics Consortium and the EU sponsored COVID-HLA-Genome (COHLAGE) have been organized to further study the influence of genetics on SARS-CoV-2 infection and immune response.

Host Genetic Factors

Elucidating host Genetics of COVID-19 holds potential for understanding both susceptibility to SARS-CoV-2 infection as well as heterogeneity in clinical presentation and severity in disease outcome. Candidate gene association studies performed during SARS-CoV-1 have provided valuable information regarding the role played by different genetic variables in influencing coronavirus infections, for example on genetic predisposition, pathogenesis, clinical course and outcome. However, large, systematic studies assessing genome-wide associations of genetic variants with coronavirus disease in humans are still lacking.

We discuss here important genetic factors that might help to explain the clinical conundrum of COVID-19:

i. HLA-linked Immune Response Genes could potentially influence COVID-19 Immunity and disease susceptibility

During COVID-19 immunopathogenesis, the released viral particles are engulfed by the antigen presenting cells (dendritic cells, B cells, macrophages) and the viral antigenic peptides are presented through the HLA (Human Leukocyte Antigen) system encoded by the most polymorphic gene dense region 'major histocompatibility complex' (MHC) of the genome (>26000 alleles)[7] (*https://www.ebi.ac.uk/ipd/imgt/hla/stats.html*). The HLA class I and II molecules select the viral epitopes to present to CD8+ and CD4+ cells respectively which initiate cellular and humoral immune responses. In various

genome wide association studies, it is also known to be the significantly linked genetic loci in many diseases including viral diseases[8]. The basic details and the biological relevance of this system is explained below:

The HLA System: The histocompatibility or the human leucocyte antigens (HLA) as they are referred to in humans are cell surface glycoproteins expressed on nucleated cells whose major function is to bind peptides within the cell and present them at the cell surface for inspection by T-cells of the immune system. They are a part of the major histocompatibility complex (MHC) that exists in most vertebrate species and whose antigens were originally defined as the most important molecules involved in the rejection of transplanted tissues, exchanged between individuals of the same species.

The HLA gene are arranged in the form of three regions: class I, class II and class III **(Fig. 2)**. Most of these are polymorphic, arranged close together and are generally inherited *en bloc* as a haplotype. The class I region is the most telomeric part of the MHC complex. Although 36 genes have been defined so far in this region, HLA-A, B and C are the most important since their products have been well defined as 'classical transplantation antigens'. They are characterized by high degree of polymorphism in

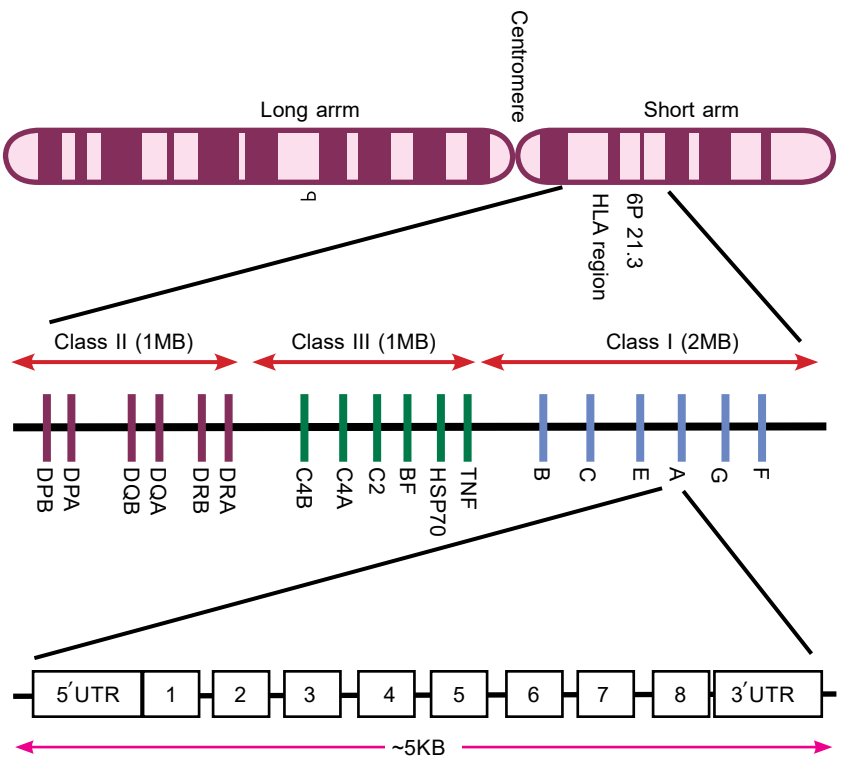

Fig 2: *Simplified gene map of the human Major Histocompatibility Complex (MHC), depicting key genetic loci on the short arm of the chromosome 6 (6p21.3).*

most vertebrate species. HLA class II region extends over 1000–1200kb with at least six subregions, termed DR, DQ, DP, DO, DN and DM.

One striking feature of the products of the classical class I and II loci is their extreme degree of polymorphism. Indeed, no other genetic loci are known to have a similar degree of polymorphism, which means that an exceptional inter- individual variability exists as far as the HLA profile of a population is concerned. The major biological function of the HLA molecules is to bind peptides for presentation to T-cells.

HLA class I molecules are expressed ubiquitously on all nucleated cells and they present *endogenously* derived antigenic peptides to CD8+ cytotoxic T-cells for inspection and elimination by mounting an appropriate immune response. The class II molecules on the other hand have a rather restricted tissue distribution being preferentially expressed on antigen presenting cells (APCs) that include macrophages, dendritic cells, fibroblasts, Langerhans cells, Kupffer cells. They present *exogenous* foreign peptides derived from viruses, bacteria, parasites or any other source (including the mismatched transplant tissues/organs) recognized as non-self by the host to the CD4+ helper T-cells.

Such functional divergence of the two specialized HLA molecules is perhaps necessary to increase and optimize efficiency of the immune system for handling a broad spectrum of peptides. Since CD4 T-cells are placed at the initial phase of the immune response and provide helper signals to both CD8+ T-cells as well as B cells, they are central to both extracellular and intracellular surveillance.

With the introduction of high throughput next generation sequencing (NGS) techniques and data having become available from diverse population groups around the world, an impressive number of over 25,000 HLA alleles has become known**.** It may be mentioned that the Indian population shows the highest genetic diversity of HLA alleles with the existence of several 'novel HLA alleles' and 'Unique HLA haplotypes'**.** The most plausible explanation given for such a diversity is the high microbial load prevalent in the sub-continent, besides the historical racial admixture, leading to novel allelic recombination events.

The question then is: Why should genetic variation in HLA genes play a role in the COVID-19 progression? Studies carried out on related viral diseases have indicated that HLA phenotypes may determine the susceptibility status of an individual against such viruses. At the population and individual level, higher diversity of HLA alleles enhance the probability to select HLA molecules with higher binding specificities to the SARS-CoV2 peptides which can compromise viral replicative fitness. For example, protective heterozygous advantage as observed for HIV infection and disease progression[8].

Immunogenetic Association

Though immunogenomic data is lacking on SARS-CoV2, reports are available on SARS CoV (closest human coronavirus to SARS-CoV2) and HLA. For example, HLA-

B*46:01, B*07:03, DRB1*12:01 and C*08:01 were found significantly associated with SARS-CoV susceptibility. In contrast, HLA-C*15:02 and DRB1*03:01 were protection conferring[9]. On the other hand, Umapathy et al[10] reported that the absence of HLA-B*46 in Indians could be protective in SARS epidemic.

More recently, Nguyen et al[11] performed a comprehensive *in silico* analysis of viral peptide-MHC class I binding affinity across 145 HLA-A, B and C genotypes for all SARS-CoV-2 peptides. Allele HLA-B*46:01 revealed the fewest predicted binding peptides, for SARS-CoV2 suggesting individuals harbouring it could be vulnerable. Conversely, HLA-B*15:03 showed the greatest capacity to present highly conserved viral peptides signifying possible cross protective T-cell based immunity through it. HLA genotypes may differentially induce the T-cell mediated antiviral response and might explain the heterogeneity of clinical outcomes.

Recently, a publicly accessible database of epitopes, which were predicted to bind any class I HLA protein across the entire SARS-CoV-2 proteome has been developed[12]. Further, Nerli and Sgourakis[13] has performed structure-based modelling of SARS-CoV-2 peptide/HLA-A02 antigen and the models generated are available on online database (*https://rosettamhc.chemistry.ucsc.edu*).

Besides immunoinformatic studies and prediction modelling, others have utilized NGS based HLA typing in COVID-19. A small cohort of 82 patients revealed significantly increased occurrence of HLA-C*07:29, and B*15:27 in COVID-19 patients as compared to controls[14]. Similarly, Iturrieta-Zuazo et al.[15] analyzed binding affinity of 66 HLA class I alleles and SARS-CoV2 peptides in the context of disease severity. They observed that patients with mild COVID-19 showed greater heterozygosity and expressed HLA alleles with a higher theoretical capacity for binding SARS-CoV2 peptides as compared to those with severe course of disease.

i. Genetic Diversity of HLA in India

India represents a region with immense geographical, cultural and linguistic diversity along with appreciably higher genetic diversity than any other region after Africa. Genetic analyses by various groups including ours have revealed unique as well as extensive diversity at the allelic, genotypic and haplotypic levels in the HLA class I and class II genes in Indian population. For example, the allele families of HLA-A2 and A33 represent great diversity and existence of unique alleles[16].

One of the 'novel allele' observed is A*02:11, which occurs frequently in the Indian subcontinent with nearly complete absence in Caucasoid and Oriental populations. Another allele A*33:03 which primarily occurs in the Mongoloid populations is also observed in high frequency in North Indians.

Similarly, high genetic diversity has been observed in several HLA class II allelic groups. Studies carried out by Prof Mehra's group at the AIIMS, New Delhi identified remarkable diversity in HLA-DR3+ extended haplotypes that predispose to autoimmunity. A good number of these were found to be 'unique' to the Indian

population[17]. Particularly, the classical caucasian autoimmunity associated AH8.1 (A1-B8-DR3) haplotype is rare in Indians and is replaced by variant haplotype(s) like AH8.2 (A26-B8-DR3), which is the most common DR3+ve haplotype here along with several others[18]. This favoured variance of AH8.2 over AH8.1 could be ascribed to the evolutionary advantage determined through distinct pathogenic challenges encountered by the Indians during evolution.

This observed diverse HLA profile could be ascribed to microbial selection pressure and/or as a consequence of the founder effect, racial admixture due to history of several invasions and geophysical as well as socioeconomic barriers resulting to the genetic drift. We anticipate that HLA diversity among Indians is suggestive of evolutionary advantage mediated through extensive repertoire of these antigen-presenting molecules in relation to disease susceptibility and resistance. Thus, we envisage a better immune response of Indians against the ongoing COVID-19 outbreak. However, this warrants in-depth studies involving comparative immune-genomic evaluation in the context of the disease severity across various populations.

Further, it is conceivable that natural killer (NK) cells are immunologically potent in controlling viral replication. These cells express Killer Immunoglobulin Receptor (KIR) molecules on their surface which regulate their activity through binding to the cognate ligands i.e. HLA-C molecules[19].

The KIR genes (16 genes with several alleles) are highly polymorphic and are categorized into activating (KIR2DS1-KIR2DS5, KIR3DS1), inhibitory (KIR2DL1-KIR2DL5, KIR3DL1-KIR3DL3) and pseudogenes (KIR2DP1, KIR3DP1) based on their molecular structure and functional influence[19]**.** Literature strongly supports the influence of HLA and KIR (independently as well as interacting partners) in various infectious and autoimmune diseases. However, there are no reports on COVID-19 so far.

In view of the ongoing COVID-19 outbreak, the completely unknown immuno-genomic correlates of protection and/or vulnerability involving polymorphic HLA and KIR alleles or haplotypic groups is much desirable in clinically well-defined cohorts involving different ethnicities.

ii. Blood Groups and COVID-19

The first such study published in the New England Journal of Medicine in June identified 'two genes' linked to respiratory failure in a sample of 1980 COVID-19 patients with severe disease drawn from hospitals across Italy and Spain[20]. Of these, one gene located on chromosome 9 coinciding with the ABO blood groups identified higher risk of disease among persons with blood group A and a protective effect for those with the 'O' blood group. Similar results were obtained by the Danish Health registry indicating least vulnerability of blood group 'O' to COVID-19, as well as a Vancouver study reporting that A and AB blood group types were at a comparatively higher risk of severe symptoms. However, it will be premature to draw any definitive conclusions

from these results unless extensive studies are carried out in other populations and the underlying mechanisms get clarified. Until that happens these would remain an academic interest and perhaps a chance finding.

iii. Toll-like Receptors: *Evidence from a Case Study*

Toll like receptors (TLRs) represent a group of pattern recognition receptors (PRRs) which initiate the innate immune response through sensing the pathogen associated molecular patterns (PAMPs). Incidentally, among all the known PRRs, TLRs are evolutionarily the most ancient group. Typically, TLRs contain three structural domains including a leucine rich repeats (LRRs) motif for pathogen recognition, a trans membrane and a cytoplasmic Toll/IL-1 receptor (TIR) domain for signal transduction through interaction with adaptors resulting in the production of various cytokines and chemokines.

Broadly, TLR signalling pathways are classified into two categories, a) myeloid differentiation primary response protein 88 (MyD88)-dependent pathway used extensively by almost all TLRs except TLR3 and b) TIR domain-containing adaptor-inducing IFNβ (TRIF)-dependent pathways which are specific for only few TLRs like TLR3 and TLR4.

In humans, 10 TLRs (TLR1-10) are known so far, of which TLR1,2,4,5,6 and 10 are expressed on cell surface and involved in recognizing microbial membrane and/or cell wall moieties, while TLR3,7,8 and 9 are expressed by the endo-lysosomal membranes and primarily recognize nucleic acids.

Severe COVID-19 can occur among younger, predominantly male patients without pre-existing medical conditions. Similarly some individuals may have primary immunodeficiencies that predisposes them to severe viral infections including by SARS-CoV-2. A case series involving two pairs of brothers, mean age of 26 (range 21-32) with severe COVID-19 from two unrelated families of Holland was published in July last year[21]. Rapid clinical whole-exome sequencing was performed to identify a potential monogenic cause. Basic genetic and immunological tests were also performed in primary immune cells isolated from the patients and family members to characterize any immune defects.

The study found a maternally inherited 4-nucleotide deletion mutation in the *TLR7* gene (c.2129_2132del; p.[Gln710Argfs*18]) of the first two brothers and a single base missense substitution (Val795Phe) in the same gene of the second pair of brothers. In both cases, this led to a significant reduction in the production TLR7 protein. By the way, TLR7 is known to trigger the production of interferons, which are the signalling proteins that are essential in defence against virus infections. In this particular case, the production of IFN-γ, a type II IFN, was decreased in patients in response to stimulation with imiquimod. It is now clear from other studies that SARS-CoV-2 infection causes reduction in the production of interferons by immune cells.

TLR7 function has so far never been associated with an inborn error of immunity. This case series has unexpectedly provided a clue that this molecule may be essential for protection from COVID-19 and that its genetic absence in homozygous or heterozygous state could be responsible for the severity of the disease in the brothers.

The study also highlights a novel avenue for the exploration of potential treatments for COVID-19 and could provide an explanation for the observed trend of higher fatalities from this disease in men than women.

iv. The COVID-19 Host Genetics Initiative

The COVID-19 Host Genetics Initiative, an international study group of eminent Geneticists, is established as a global data resource for genetic architecture of COVID-19 with hundreds of studies already registered so far[22,23]. Ithas released new dataset to suggest that a region on chromosome 3 shows significant association with severe COVID-19 with an odds ratio for requiring hospitalization to be 1.6. The gene encodes for a protein that seemingly influences interaction between two very important cells of the immune system, namely the Dendritic cells and T-cells, and thus may directly control clinical course of the disease. This has been confirmed in a Genome wide association study led by Kenneth Baillie of the University of Edinburgh on over 2000 critically ill COVID-19 patients suggesting at least half a dozen additional gene variants.

Of the additional genes linked to severe COVID-19 in the Edinburgh study is the IFNAR2 that codes for interferon[24] (also covered in section on other genes), which acts as a powerful messenger that influences the development of innate and the adaptive immune responses against viral infections. Increased expression of this gene could reduce the odds of severe COVID-19 and this finding raises hopes of better outcome for ongoing trials of interferons as a plausible COVID-19 treatment. The other five genes act in pairs and influence two biological mechanisms: one set determines innate immunity driven antiviral defenses 'early in disease' and the other inflammatory lung injury that occurs 'later in disease' course and is life-threatening. Again, these claims must be tested in large scale clinical trials before entering clinical practice.

v. Regeneron Genetics Center Study

In the most comprehensive trans-ancestry sequencing study of COVID-19 involving over half a million individuals, researchers at the Regeneron Genetics Center in New York have focused on three main components namely, susceptibility to SARS-CoV-2 infection, disease severity as determined by hospitalization and the third of patients who required ventilation or had passed away. This mega attempt confirms the involvement of chromosome 3 genes, but not the ABO blood groups suggesting that it may be a false positive. Further, they identified 'three additional novel genes 'expressed on a) chromosome 1 and found mostly in individuals of African ancestry, b) Chromosome 16 and c) chromosome 22, the last of which also influences the IFNAR2 gene.

vi. Other Genes

a. **IFNAR2:** Since critical illness caused by COVID-19 is qualitatively different from mild or moderate disease, even among hospitalized patients, a study carried out at the University of Edinburgh under Kenneth Baillie evaluated the genetic mechanisms involved in precipitating critical illness.[24] Novel genome-wide strategy was employed because of its potential to reveal more robust information on possible therapeutic targets to modulate the host immune response to promote survival. The study named GenOMICC (Genetics of Mortality in Critical Care) included over 2200 critically ill patients drawn from over 200 intensive care units across UK. Significant genome wide associations were observed on various loci as under:

   i. chromosome 12q24.13 (rs10735079, $p=1.65\times10^{-8}$) in a gene cluster which encodes antiviral restriction enzyme activators (OAS1, OAS2, OAS3).
   ii. chromosome 19p13.2 (rs2109069, $p=2.3\times10^{-12}$) near gene encoding tyrosine kinase 2 (TYK2)
   iii. chromosome 19p13.3 (rs2109069, $p=3.98\times10^{-12}$) within the gene for dipeptidyl peptidase 9 (DPP9) and
   iv. chromosome 21q22.1 (rs2236757, $p=4.99\times10^{-8}$) in the interferon receptor gene IFNAR2.

A causal link was observed for low expression of IFNAR2 and high expression of TYK2, to the life threatening disease. These results suggest a genetic link to key host antiviral immunity and inflammatory organ damage in COVID-19. However, translational and clinical utility of these observations will require large scale randomized clinical trials.

b. **Angiotensin converting enzyme 2:** Since ACE2 is the primary cellular receptor for SARS-CoV-2, some investigators have tried to investigate a possible relationship between *ACE2* polymorphisms and COVID-19 severity. Further, the observed increased severity of COVID-19 in males could also be associated with *ACE2* polymorphisms and expression levels.

Incidentally, the expression of *ACE2* has been reported to be higher in men than women[25,26]. This has made some investigators to speculate that the pattern and density of *ACE2* expression in different anatomical locales could influence sex-based differences in disease severity. Since ACE2 is encoded on the X-chromosome, males express only a single ACE2 variant on all cells. Thus if men harbour an *ACE2 genetic variant* that is more permissive for SARS-CoV-2 infection, all of their cells will express this risk variant. On the other hand, females with two X-chromosomes will express a mosaic pattern of ACE2 and are less likely to develop critical illness.

c. **TMPRSS2:** The serine protease TMPRSS2 is the another host protein involved in SARS-CoV-2 infection, whose polymorphism could influence the severity

of COVID-19. Recently, a SNP associated with higher expression of TMPRSS2 was identified which could potentially influence SARS-CoV2 susceptibility[27]. Further, many other SNPs in this gene were observed in Italian cohort which could affect expression levels[28]. As of now, only a handful of studies have been done using the single nucleotide polymorphism (SNP) approach, and hence the data is preliminary.

vii. Epigenetic Factors

These include environment, lifestyle and food habits that may also play a beneficial role for countries such as India. The extensive geographical range observed throughout across various regions and states of India e.g. Himalayas mountains to beaches and deserts could result in variable climatic conditions along with diverse lifestyle habits and food habits. Interestingly, reports from Ayurveda and other medicine disciplines suggest useful effects of Indian spices in strengthening immune homeostasis. A classic example most commonly used food ingredient is of turmeric, which contains curcumin (active polyphenol) with several known antioxidant, anti-bacterial, anti-inflammatory, and wound healing properties. Literature suggests its convincing impact in treating cancers, arthritis, cardiovascular and inflammatory bowel diseases[29]. Likewise, several other spices as food ingredients are used consistently. Overall, these epigenetic, environmental and lifestyle dependent factors could influence immunological vulnerability against COVID-19.

COVID-19 Therapy

Adoptive cell therapy using viral-specific T-cells has been successfully used to treat life-threatening viral infections, supporting the application of such an approach for COVID-19. Basar et al[30] bioRxiv preprint doi: *https://doi.org/10.1101/2020.09.15.298547* this version posted September 15, 2020) were able to successfully expand T-cells from the peripheral blood of COVID-19 recovered donors, directed specifically against structural SARS-CoV-2 proteins including the receptor binding domain of Spike. The immuno-therapy approach could be used to treat critically ill COVID-19 patients.

However since corticosteroids are increasingly used to treat these patients and could be a potential hurdle to the beneficial effects of immunotherapy, the investigators developed an efficient strategy to genetically modify and inactivate the glucocorticoid receptor gene, NR3C1 in SARS-CoV-2 CTLs through CRISPER-Cas9 gene editing.

Convalescent Plasma

In the absence of other effective therapies, convalescent plasma, first tested as a passive immunisation therapy against diphtheria, tetanus and other viral diseases in the late 19th century has been used to treat COVID-19 also. The rationale behind its use is that it is a ready-made source of SARS-CoV-2 specific antiviral neutralizing antibodies which could exert a potent therapeutic effect in patients with COVID-19. Additionally,

anti-inflammatory cytokines, defensins, pentraxins, and other immunomodulatory proteins might help in alleviating systemic inflammatory response syndrome, which is the main pathophysiological basis for acute respiratory distress syndrome and mortality from covid-19 related pneumonia.

Current evidence suggests that a proportion of the recovered COVID-19 patients are expected to raise receptor binding domain specific antiviral antibodies and the convalescent plasma collected from them could be potentially useful for treating patients, particularly those who fail to raise timely neutralizing antibodies of good titre.

However, several questions remain unanswered. For example, what should be the effective titre of neutralizing antibodies in the infused convalescent plasma? Would a relatively younger donor be more appropriate? What should be the optimal time for plasma donation and what severity level of patients are more likely to be benefitted?

Several countries have given regulatory approval for use of convalescent plasma as a treatment option for COVID-19. However, a systematic review on the studies carried out so far has revealed variable results with no real benefit on reduction in mortality, hospital stay or the viral load in such patients[31]. Two randomised controlled trials, one from China[32] and the other from Holland[33] had to be stopped prematurely because of the faulty design**.** Nevertheless both these studies failed to find a mortality benefit. Further, the Dutch study raised uncertainties about the pretransfusion antibody status of patients as a potential factor in identifying appropriate candidates who might benefit from convalescent plasma treatment.

A multicentre randomized, controlled phase 2 clinical trial conducted in India as PLACID trial, involving hospitalized adults with confirmed moderate COVID-19 patients failed to show an effect of convalescent plasma (CP) on progression from moderate to severe disease or mortality[34]. Designed and sponsored by the Indian Council of Medical Research (ICMR), the study had 464 adults from 39 tertiary care hospitals across India, divided in two groups of 235 patients given two doses of 200 ml of CP and a group pf 229 given best standard of care only. The authors concluded that *a priori* measurement of neutralising antibody titers in donors and participants might help in better ascertaining the role of convalescent plasma in the management of covid-19.

Because of the questionable outcome, the enthusiasm of clinicians in using convalescent plasma as a treatment option for COVID-19 has considerably subsided. At best, it may show some promise if given early on in the infection, when the need for such a therapy is actually debatable.

Vitamin D and COVID-19

Evidence from observational studies is accumulating, suggesting that the majority of deaths due to SARS-CoV-2 infections are statistically attributable to vitamin

D insufficiency and many of these could potentially be prevented by vitamin D supplementation. Radujkovic and coworkers[35] were among the first to report an association between vitamin D deficiency (25(OH)D < 12 ng/mL) or insufficiency (25(OH)D < 20 ng/mL) and death in a cohort of 185 consecutive symptomatic SARS-CoV-2-positive patients admitted to the Heidelberg Medical University Hospital in Germany. Of the 118 patients (64%) those with vitamin D insufficiency, at least 16 died of the infection. Based on population based analysis, these investigators stated that a very high proportion of COVID-19 deaths (~87%) in Germany could statistically be attributable to vitamin D insufficiency and that many of these could be avoided by vitamin D supplementation.

The mechanism underlying such an association is not fully clear, although cytokine imbalance due to Vit D insufficiency, namely high concentration of pro-inflammatory cytokines and low levels of anti-inflammatory cytokines has been proposed in some studies[36,37].

Randomized clinical trials could throw more light on the role of Vitamin D in COVID-19. Nevertheless, experience from other acute respiratory infections including asthma and chronic pulmonary disease point towards a beneficial effect of targeted Vitamin D3 supplementation in COVID-19.

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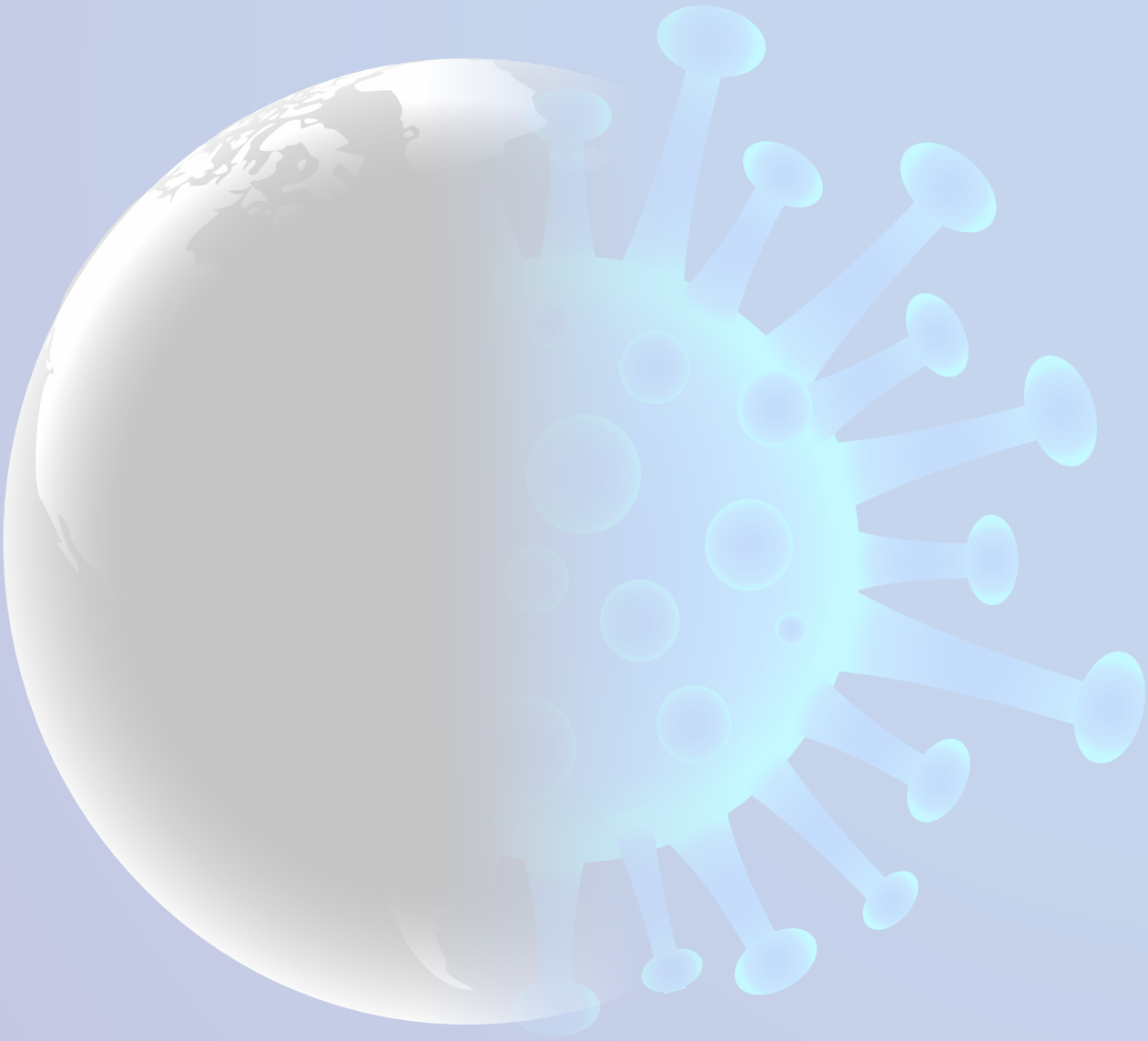

CHAPTER 6

INTERNATIONAL COVID-19 VACCINE DEVELOPMENT AND CLINICAL TRIALS

Vaccine development has always been a tough and arduous task, typically requiring 15 to 20 years. In spite of ongoing work on malaria, tuberculosis and leprosy vaccines for several years, we have still not succeeded. Vaccine development initially involves pre-clinical phase of laboratory testing and animal studies before they are tested in human clinical studies. The pre-clinical development component may take several years before a candidate vaccine become available for human testing.

The primary expectation from any vaccine is that it should be safe, immunogenic and efficacious. Phase I human studies are carried out in a small number of healthy individuals to demonstrate safety of the vaccine candidates as per pre-determined norms and may take a few months to be completed. Such vaccine candidates that are proved to be safe in Phase I are taken to Phase II trials in individuals at some risk of contracting that disease with the primary objective of assessing the ability of the vaccine candidates to induce appropriate immune response against the specific pathogen. In addition to evidence of immunogenicity, long term safety of the vaccine candidates is also assessed in Phase II studies which may last for several months to a year. Finally, the ability to prevent the occurrence of disease [described as efficacy] is tested in Phase III clinical trials which normally involve several thousand individuals who are at high risk of developing the disease caused by the pathogen against which the vaccine is being developed. Generally, the results of such trials become available after several years.

Regulatory bodies examine the evidence and take an appropriate decision on granting licensure to the vaccine in their country. This step-by-step vaccine evaluation approach explains the long waiting period for newer vaccines to be made available to the people after ensuring that they are safe, immunogenic and efficacious.

Significant advancements and developments have happened in the area of bio-medical technology and instrumentation over the last few decades which have

resulted in reduction in time required for candidate vaccine development. High quality adjuvants have become available that are believed to significantly improve the potency of newer vaccines.

Vaccines against COVID-19

Right from the time of the onset of the pandemic in early 2020, there have been predictions and claims that COVID-19 vaccine is likely to be available in 18-24 months or even earlier. However, it was realized that if the goal is to introduce a COVID-19 vaccine as an urgent public health intervention during the current pandemic, novel approaches will be necessary. One approach could be to skip the component of animal testing. For example, in case of Seattle, USA based COVID vaccine trial, the experimental vaccine was administered to laboratory mice concurrently, with the initiation of the human trials and recruitment of participants. Even after criticism and questions raised by biomedical ethicists for such a shortcut approach, the trial was initiated with a firm regulatory backing.

Another approach by which the human testing phase of candidate vaccines can be hastened is by designing Phase IIb trials. The design of such trials requires larger sample size and the goal is to assess the level of immunogenicity as a surrogate for its efficacy. The study period of such a trial can also be extended to accommodate early assessment of efficacy. Certainly, the need for employing novel approaches to generate optimum evidence for decision making by the in-country regulators regarding fast-track product licensure during the pandemic scenario is being increasingly discussed.

There has been some recent history of fast-track vaccine development. Ebola virus was first identified in 1976 and it caused several outbreaks until 2014, which were quickly and effectively controlled. Hence commercial vaccine manufacturers showed no enthusiasm to advance the experimental Ebola vaccines through clinical trials. However, following the 2014 Ebola outbreak, several vaccines which were previously tested only in animals are now being fast-tracked into Phase I, II and III human clinical trials. Ebola Zaire was the strain of the Ebola virus that was responsible for the 2014 outbreak; and all of the vaccine candidates being advanced are designed from that strain to prevent infection. If successful, this vaccine construct can be quickly applied to the other Ebola virus strains.

The GSK adenovirus vector vaccine and a Merck, New Link Genetics recombinant vaccine are the other two examples being tested in a single Phase 2 trial in Liberia in those at risk for Ebola Virus Disease (EVD). Other vaccine candidates that are also being currently evaluated include Thomas Jefferson University's Vaccine Centre's vaccine candidate and the one developed by Johnson and Johnson USA. Planning for phase II and III of Ebola vaccines is ongoing.

Typically, for a very rapidly spreading acute communicable disease that shows some evidence of intermediate to high case fatality, the primary preventive tool that one can hope for, is a vaccine in the absence of a known and effective curative

intervention. The experience of working on Ebola and MERS vaccines has been very useful for the global scientific community, because it already provided them with a template for understanding the basics of a rapid response for developing vaccines during global health emergencies.

Most COVID-19 vaccine development activity is in North America with 36 (46%) developers of the confirmed active vaccine candidates as compared with 14 (18%) in China, 14 (18%) in Asia (excluding China) and Australia, and 14 (18%) in Europe[1] (**Fig. 1**).

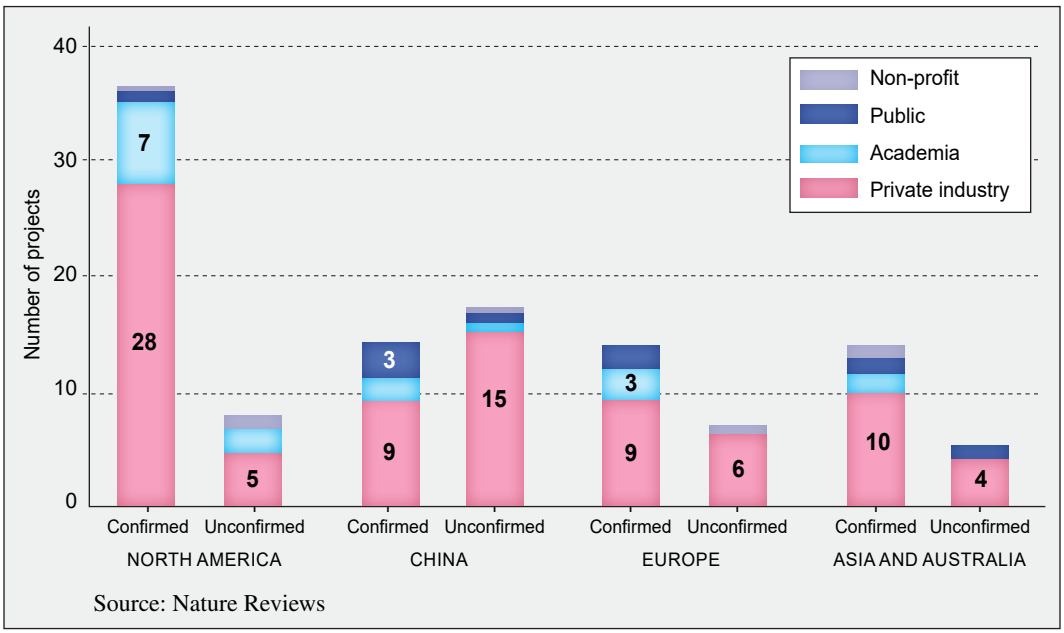

Fig. 1: *Profile of COVID-19 vaccine developers by the type and geographic location. For partnerships, the location is that of the lead developer. *Excluding China.*

Certain important and critical questions that have been considered in deciding the type of vaccine to be developed against COVID-19 include the quantum and type of immune response likely to be elicited by the candidates, the length for which the protection would be offered, the presence of antibodies to natural exposure to coronavirus in the community and what can be considered as reliable correlates of protection[2]. In the current scenario when there is relative haziness around understanding the relative contribution of humoral and cellular immunity in COVID-19 and also the lack of adequate knowledge about the protective role and longevity of the neutralizing antibodies as well as those that bind to spike proteins, scientists all over the world are trying a variety of approaches for vaccine development. Three critical targets or milestones for SARS-CoV-2 vaccine development include antigen selection and engineering, preclinical challenge studies in non-human primate models, and demonstrating the immune correlates of protection[3].

Using the whole virus, either killed or live attenuated would always be the first option for the target of achieving a more complete immunological response equivalent to a natural infection. However, there could be a possibility of reversion in case of live attenuated vaccines, inadequately protective immunological response due to inactivation processes and higher toxicity resulting from the process of inactivation. The focus is therefore shifting to newer approaches that do not require whole virus cultivation with the ultimate aim of designing a candidate vaccine that would yield a quicker immune response in the host during outbreak situations.

Use of DNA, mRNA, protein or sub-unit vaccines are expected to bring down the toxicity profile of a candidate vaccine to a large extent, although these might also negatively affect their immunogenicity. In the recent times, scientists have started giving a lot of emphasis on the use of viral vectors as vaccine delivery platforms. Although adenoviruses are the front runners in this area, other viruses such as measles virus, vesicular stomatitis virus, modified vaccinia Ankara and adeno-associated virus have also been evaluated in other vaccine candidates under development[4].

In addition to the issues discussed above for an effective vaccine design, there are other impediments in rapid vaccine development in an outbreak scenario and these include delays due to undefined vaccine targets, cost of manufacturing and limited production capacity of traditional vaccine development approaches. Globally, the aim to develop newer platforms that will enable fast track development of cost-effective vaccines and with the global level of scalability.

Even with the above discussed background challenges, the progress on COVID-19 vaccine development has been truly unprecedented. The genetic sequence of SARS nCov2 was released on January 11, 2020, following which vaccine development platforms were developed on fast track and the first human COVID vaccine trial was started on 16th March 2020 in a record time of just a little more than two months[5]. The first six vaccine candidates entering phase I clinical trials to assess their safety and immunogenicity, included those based on mRNA, adenoviral vector 5; chimpanzee adenoviral vector ChAdOx1, DNA, a lentiviral vector and artificial antigen-presenting cells or aAPC[6].

Various vaccine candidates in clinical trials have been designed to express the COVID-19 spike (S) glycoprotein which is able to neutralise the COVID causing coronavirus and prevent attachment to the human angiotensin converting enzyme II (ACE2) receptor, known to be the co-receptor for viral entry of SARS-CoV-2[7].

A computational model developed in the US simulating the spread of COVID-19 coronavirus and vaccination showed that at 60% efficacy and 100% coverage, there was an opportunity to reduce the peak by >99%. With vaccine efficacy threshold rising to 70% or 80%, the same effect could be achieved with a drop in coverage to 75% and 60% respectively. It was hypothesized based on the results of this modelling study that with vaccine efficacy of at least 60% and coverage of 100%, it is possible to stop the pandemic and with a vaccine efficacy of 80% and coverage of 75%, the

peak could possibly be reduced by 85 to 86%. The study concluded that a vaccine with an efficacy of at least 70% could prevent the epidemic and with the efficacy touching 80% and above, it might help to even extinguish an epidemic without any other measures[8]. It therefore appears that it would be important to have a COVID-19 vaccine with a higher efficacy so as to achieve reasonable control of the pandemic.

Phase I and II Clinical Trials

Although traditionally vaccine development, clinical evaluation and marketing is a long process requiring several decades of work; there is an increasing realization that in case of serious global public health emergency situations, some practical and proactive approaches are required to be taken to fact track vaccines under development to combat them[9]. Such approaches would include a more meaningful engagement and flexible as well as adaptive approach by ethics committees and regulatory bodies in providing timely approvals for human clinical trials as well as product licensure eventually.

It is also critical to create platforms for fast-track development and processing of genetically modified vaccine candidates and availability of centralized, well-supervised facilities for testing immunogenicity under strict quality control. There is a strong need for designing adaptive study designs to enable early licensure and marketing of vaccines within the available regulatory framework of the respective countries. For this, some background work will have to be done, clinical trial sites that are capable of conducting Phase I, II and III trials will have to be developed with active engagement of local communities.

It is equally important to create a feeling of trust and confidence in the community about the ongoing vaccine development and trial initiatives through a systematically designed and transparent process of communication and constant dialogue with key stakeholders and the community. While planning COVID-19 vaccine trials, it would be important to select a multi-centric trial design with a suitable placebo control that would demonstrate adequate proof of desired levels of safety and efficacy through long term protection from the disease and possibly protection from development of severe form of the disease[10].

Table 1 summarizes the vaccines that are currently in Phase I and II human clinical trials[11, 12].

Results of Phase I/ II Clinical Trials

i. Inactivated COVID Vaccine

Phase 1 and 2 clinical trials of an inactivated COVID-19 vaccine were conducted among 96 and 224 healthy adults respectively in Henan Province of China. In the Phase I study, a 3-dose dose-escalation design was used and the Phase 2 study was a 2-dose variable interval study. Volunteers developed adverse

Table 1: COVID-19 vaccines in Phase I and II clinical trials

Vaccine platform	Process and technique	Phase of human clinical trial	Company/ Industry
Inactivated vaccines	Formalin-inactivated and alum-adjuvanted vaccine candidate	Two Phase I and II trials	Sinovac in Jiangsu, China
	inactivated SARS-CoV-2 vaccine	Phase I and II trials	Sinopharm in Shanqui, China
DNA and MRNA vaccines	double stranded DNA, INO-4800	Phase I trial	InovioPharmaceutiocals, USA
	4 mRNA vaccine candidates, BNT162a1, BNT162b1, BNT162b2, and BNT162c2	Phase I trial	BioNTech and Pfizer, USA
	mRNA vaccine, mRNA-1273	Phase I trial	Moderna, USA
Non-replicating viral vectors	Adenovirus type 5 vector	Phase I and II trials	Cansino Biologicals, Wuhan, China
	Chimpanzees Adeno virus vector ChAdOx1 nCoV-19	Phase I and II trials	Oxford University and AstraZeneka, UK
Protein subunits	SARS-CoV-2 recombinant Spike nanoparticle vaccine with and without Matrix-M adjuvant	Phase I trial	Australia
	NVX-CoV2373	Phase I and II trials	Novavax, USA
	Recombinant adenovirus type 26 (rAd26) vector and a recombinant adenovirus type 5 (rAd5) vector – Sputnik V	Phase I and II trials	Russia
Antigen presenting cells	inactivated artificial antigen-presenting cells expressing conserved structural and protease epitopes of SARS-CoV-2	Phase I trial	China
pre-approved vaccines:	BCG	Phase III trial, 4170 participants	Australia
	BCG	Phase III trial, 1500 participants	The Netherlands
	BCG	Phase III trial, 500 participants	South Africa
	BCG	Phase IV trial, 1800 participants	USA

1. Adopted from Al-Kassmy J, Pedersen J, Kobinger G. Vaccine Candidates against Coronavirus Infections. Where Does COVID-19 Stand? Viruses. 2020 Aug 7; 12(8):861. doi: 10.3390/v12080861. PMID: 32784685 and Asher Mullard. COVID-19 vaccine development pipeline gears up. Lancet, The, 2020; 395: 1751-1752, doi: 10.1016/S0140-6736(20)31252-6. PMID: 32505245.

2. Several other countries have planned trials on oral polio vaccine (OPV), and the measles-mumps-rubella (MMR) vaccine.

reactions albeit at low rates and demonstrated desirable immunogenicity as well as neutralizing antibodies at 14 days[13].

There are four ongoing inactivated vaccine trials in China, evaluating different optimization strategies and use of an adjuvant which may demonstrate some evidence and insight on the utility of inactivated platforms against COVID-19 disease. Immunogenicity of a whole virion inactivated SARS-CoV-2 vaccine [BBV152] developed on a Vero cell platform and adjuvanted with aluminium

hydroxide gel was evaluated in mice, rats and rabbits at antigen concentration levels of 3 and 6 micrograms[14]. High antigen binding antibody titers and neutralizing antibody titers were observed. The double-blind, multicentre, randomised, controlled phase 1 trial to assess the safety and immunogenicity of BBV152 at 11 hospitals across India[15] demonstrated tolerable safety outcomes and enhanced immune responses and the candidate is being currently tested in Phase II and III trials in India.

In another study, using an inactivated vaccine in the mouse model, protection from challenge was reported against both SARS and MERS; however, the protection was only partial and short-lived in ferrets and humans[16]. It therefore appears that if protection is dependent on neutralizing antibodies, several boosts would be required for long term protection against COVID-19 among humans.

Some inactivated COVID-19 vaccines have shown promising results in Phase I/ II clinical trial in human beings and some candidates have already moved to Phase III evaluation. This is in line with the previous vaccine trials using inactivated viruses. However, this platform might prove to be an inferior choice as compared with other strategies.

ii. DNA Vaccines

Although, DNA platforms have been reported to render protection against SARS and MERS in animal studies, a combination approach involving DNA and protein resulted in better response as reported in a study employing non-human primate as the animal model[17]. The SARS-DNA (VRC-SRSDNA015-00-VP) and the MERS-DNA (GLS-5300) vaccines produced both T-cell responses and neutralizing antibody responses. However, it has been suggested that MERS-DNA might not be able to elicit an optimum neutralizing response of 50%[18]. It appears that a prime-boost model employing a DNA platform combined with a different platform might elicit better immune response and protection. Presently, there are only few COVID-19-DNA vaccine candidates in clinical trial evaluation. However, their response is somewhat similar to that seen in case of SARS or MERS.

iii. mRNA Vaccines

The mRNA-based vaccines are promising recent developments in the area of vaccine production[19]. These are believed to be a rapid and versatile platform. The perceived advantages of mRNA vaccines include their ability to induce a potent immune response and minimize vaccine associated risk of infection and insertion-induced mutagenesis. Another attractive feature is that such vaccines have the potential for rapid and large-scale production. However, their limitations include instability as compared to the DNA vaccines and the high manufacturing costs.

Nevertheless, evidence on the superiority of this platform over others is yet to be fully established.

In a record time of 69 days, the mRNA1273-COVID-19 vaccine entered the phase of human clinical trials. This lipid nanoparticle encapsulated (LNP) mRNA vaccine encoding the full length, stabilised spike (S) glycoprotein, was considered safe enough to skip pre-clinical evaluation and found direct entry in phase I human clinical trials[6,19].

In animal studies, mRNA-1273, developed by Moderna, USA induced both potent neutralizing antibody and CD8 T-cell responses. It also demonstrated protection against SARS-CoV-2 infection in mice with no immune-pathological reaction in lungs and noses of the mice[20]. Similarly, a vaccine developed by the Beijing Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, China and named as ARCoV is a lipid nanoparticle-encapsulated mRNA (mRNA-LNP) encoding the receptor binding domain (RBD) of SARS-CoV-2. It elicited robust neutralizing antibodies against SARS-CoV-2 as well as a Th1 cellular response in mice and non-human primates following intramuscular administration. Two doses of ARCoV provided protection in the challenge studies. Also, ARCoV in its liquid formulation is stable at room temperature for at least 1 week and is being evaluated in phase 1 clinical trials[21].

Two different groups are evaluating mRNA-vaccines in clinical trials (mRNA-1273 and BNT162). In a phase 1, dose-escalation, open-label study, mRNA-1273 was administered in a dose of 25 μg, 100 μg, or 250 μg to 15 participants at each dosage level; each receiving two vaccinations at an interval of 28 days. After the first vaccination, antibody responses were higher with higher dose and the titers further increased after second vaccination. After the second vaccination, neutralizing antibodies were identified in all participants matching almost to patients in convalescent phase. Adverse events were manageable and three participants (21%) in the 250 μg dose group reported one or more severe adverse events. Anti-SARS-CoV-2 immune responses were reported in all participants, with no significant safety issues[22].

The mRNA-1273 is currently in a Phase 2 clinical trial with anticipated fast track movement to Phase 3 efficacy evaluation.

iv. Adeno-virus Vector based COVID Vaccines

Among the viral vectors, adenovirus-based vaccines (ChAdOx1 and Ad5) have been previously used platform and have shown protection in mice[23]. In humans, the ChAdOx1-MERS-S vaccine candidate generated a long-lived T-cell and neutralizing antibody response even after one injection. However, concerns have been raised about the protective value of this candidate in the community wide application.

Two adenovirus-based vaccines are currently being evaluated against COVID-19 (Ad5-nCoV and ChAdOx1-nCoV). In the observations made so far, neutralizing antibodies were detected in up to 75% of participants creating a hope for a promising candidate[24]. Sub-optimum response due to pre-existing immunity to adeno viruses in humans is a concern in case of this platform. It is anticipated that the vaccine induced response may show geographic variations due to differential levels of circulation of adenovirus serotypes[25].

In a phase 1/2 randomised trial, 1077 healthy adult volunteers from UK received one injection of chimpanzee adenovirus-vectored COVID-19 vaccine and they were all followed for 28 days. Local and systemic adverse events were tolerable and manageable by basic symptomatic treatment with drugs like paracetamol. No serious adverse events were reported. In more than 90% of participants, neutralising antibodies developed which sustained for up to 8 weeks post vaccination. The second-dose resulted in strong neutralising responses, and few mild adverse events[26]. This vaccine candidate has now moved into Phase III testing.

A phase 2 randomised trial in Wuhan, China employed one injection of non-replicating adenovirus-vectored COVID-19 vaccine at two dose levels, [1×10^{11} or 5×10^{10} viral particles per mL] against a placebo with a follow-up of trial participants for 28 days[27]. The study enrolled 508 healthy COVID-19 unexposed adults. Adverse events such as fever, fatigue, headache, or local site pain occurred by day 28 in 294 (77%) of 382 vaccines and 61 (48%) of 126 placebo recipients. No serious adverse events occurred. Seroconversion was observed in >96% participants, 85% generated neutralising antibodies, while T-cell responses were observed in more than 90% participants. Encouraging results of this trial have also taken this vaccine candidate in Phase III testing.

In two open, non-randomised phase 1/2 studies at two hospitals in Russia, healthy adult volunteers were enrolled in a Phase I/ II study of a two-component vaccine, consisting of recombinant adenovirus type 26 (rAd26) vector and a recombinant adenovirus type 5 (rAd5) vector, both carrying the gene for SARS-CoV-2 spike glycoprotein[28]. Eighteen persons were enrolled in Phase I and 20 in Phase II. Both vaccine formulations were found to be safe and well tolerated. Manageable adverse events were noticed with no report of any serious adverse event. T-cell response was observed on day 28 post-vaccination and on day 42, while 100% showed sero-conversion. The candidates were recommended for Phase III evaluation and have moved into Phase III trials.

At this time, adeno-vector based COVID-19 vaccines have moved rapidly to more advanced phases of clinical trials and are considered the front-runners. Adenovirus vectors seem to be capable of generating humoral, cellular, and innate responses and are believed to have high potential in COVID-19 scenario.

Although the earlier trial results are encouraging, it is important that the proposed phase 3 trials are conducted rapidly and are large enough to determine efficacy in various sub-populations that are likely to be targeted for vaccination. It will be important to decide whether a single dose would be sufficient, particularly in older adults, or a booster dose might be necessary. Duration of clinical protection and ability to generate cell mediated immune response with a two-dose regimen will also have to be determined[29].

Additionally, the MVA vector also provided promising pre-clinical data, with different groups showing a protective response against both SARS and MERS in the mouse model[30]. In humans, although the MVA-MERS vaccine generated both T-cell responses and neutralizing antibodies in majority of the participants, the response decreased over the six months[31]. Currently, there are no active MVA-COVID-19 vaccine trials.

v. Other Approaches

Alternative platforms of live-virus vaccinations including the BCG vaccination are being evaluated globally. It is possible that the innate immune system can show adaptive response following exposure to infective pathogens or vaccinations which is similar to the adaptive immune response[32].

The BCG vaccine has previously been linked to several non-specific benefits by mounting a more efficient cytokine response. A study in 2011 found that upper respiratory infections were significantly decreased in an elderly population following BCG vaccination. Since a similar infection of the upper respiratory tract is seen in COVID-19, the use of BCG vaccine against SARS-CoV-2 might have some promising results.

Similar non-specific effects of OPV and MMR vaccinations have been reported and high MMR vaccination coverage has been linked to few COVID-19 deaths. A major advantage of this approach is that the protective effect of BCG vaccine is currently being evaluated in several Phase III/IV trials. If a beneficial tendency is seen, no further evaluations will be necessary before implementation. Whether or not this non-specific effect is sufficient remains to be seen.

Results of Phase II/ III Trials and Candidates Closer to Licensing and Human Use

Several thousand volunteers were enrolled by August 2020 in several phase 3 COVID-19 vaccine trials all over the world including those in USA, the UK, United Arab Emirates, Morocco, Argentina, Peru, Brazil, Indonesia, Russia, China, India and South Africa. Initial evidence of safety and immunogenicity is likely to emerge by the end of December 2020[32]. Some leads on safety data would also be available, but more accurate efficacy data would be available by the second or third quarter of 2021[33].

Vaccines produced by Biotech company Moderna in Cambridge, Massachusetts; by the University of Oxford and the company AstraZeneca, based in Cambridge, UK; and that by the Biotech company BioNTech of Mainz, Germany, in collaboration with Pfizer entered Phase III trials in humans a few months back. Among the Chinese vaccines, Sinovac has already initiated a phase III trial of its inactivated vaccine in Brazil and Sinopharm's inactivated vaccines will be tested in Phase III trial in the United Arab Emirates (UAE). However, although CanSino is initiating phase III trial of its candidate, its use will be restricted to military[34]. **Table 2** summarizes data on vaccines under trial.

Nearly six Chinese vaccine candidates have made a significant progress. CanSino's vaccine tweaked from a common-cold virus, Sinopharm's two inactivated vaccines

Table 2: COVID-19 vaccines in Phase III clinical trials

Company/ Industry	Platform	Vaccine candidate	Vaccine trial sites	No. of participants	Clinical trial number
The Gamaleya National Research Centre for Epidemiology and Microbiology; Academy of Military Medical Sciences	Adenovirus 5 vector and adenovirus 26 vector	Sputnik V	Russia	40,000	NCT04530396
AstraZeneca; University of Oxford	Chimpanze adenovirus	ChAdOx1/ AXD1222	UK; India; Brazil, South Africa; USA	30,000	NCT04516746
Moderna; National Institutes of Health	RNA	mRNA-1273	USA	30,000	NCT04470427
Pfizer; BioNTech	RNA	BNT162b1 and BNT162b2	USA	44,000	NCT04368728
The Janssen Pharmaceutical Companies of Johnson & Johnson	Adenovirus 26 vector	Ad26.COV2.S	USA; Argentina; Brazil; Chile; Columbia; Mexico; Peru; Philippines; South Africa; Ukraine	60,000	NCT04505722
CanSino Biologics; Academy of Military Medical Sciences	Adenovirus 5 vector	Ad5CoV	China; Pakistan	40,000	NCT04526990
Sinovac Biotech	Inactivated virus	CoronaVac	Brazil; Indonesia	9,000	–
Sinopharm; Wuhan Institute of Biological Products	Inactivated virus	–	The United Arab Emirates; Bahrain; Peru; Morocco; Argentina; Jordan	21,000	–
Sinopharm; Beijing Institute of Biological Products	Inactivated virus	BBIBP-CorV	The United Arab Emirates	5,000	–

Adopted from Gregory A Poland, Inna G Ovsyannikova, and Richard B Kennedy. SARS-CoV-2 immunity: review and applications to phase 3 vaccine candidates Lancet. 2020; 396 (10262): 1595–1606.doi: 10.1016/S0140-6736(20)32137-1 PMID: 33065034.

candidates and Sinovac's inactivated vaccine are the current leaders. However, Chinese vaccines are facing challenges during the phase III trials because of the requirement for recruiting several thousand individuals. The Chinese vaccine companies are required to test their vaccines elsewhere in the world because the Chinese COVID outbreak is largely under control and it will not be possible to find trial sites with enough study end-points in the post-vaccination period. Additionally, Chinese vaccines might face additional scrutiny because of the relative lack of transparency of the regulatory system in China and the historical difficulties encountered with defective diphtheria, tetanus and whooping cough vaccines[34].

Sputnik V Adeno Virus Vector Vaccine from Gemalaya

Sputnik V became the world's first SARS-CoV-2 vaccine to be released for human use even before the completion of Phase III clinical trial. This has naturally raised many questions. The Russian vaccine has been developed from the human adenovirus Ad5 and Ad26 vectors. A similar candidate, Oxford vaccine has employed ChAdOx, a chimpanzee's vector. The claim of efficacy of Sputnik V has been based on unpublished data from two small phase II trials, which have documented that all 76 volunteers developed high titers of SARS-CoV-2 specific antibodies and good cellular immunity[28].

Nevertheless, whether this immune response to SARS-CoV-2 is adequate enough to protect against COVID-19 is not fully clear. It is also not known whether the vaccine is safe. Although no "serious complications or side effects" have so far been reported in the phase II trials, these had included only a small number of healthy participants. Data on vaccine safety in large number of individuals is not available.

A concern has been raised about the vaccine induced SARS-CoV-2 antibodies having the potential to worsen infection or cause antibody dependent enhancement of the disease. Any negative effect of Sputnik V vaccine is likely to go beyond the people actually receiving it and affect the COVID vaccine program at large[35]. An ineffective, or unsafe vaccine has the potential to affect the confidence in the vaccine program and uptake of even other vaccines that are subsequently approved and could result in global vaccine hesitancy.

AstraZeneca Oxford Adeno Virus Vector Vaccine

In a trial entitled "Phase III Randomized, Double-blind, Placebo-controlled Multicentre Study in Adults to Determine the Safety, Efficacy, and Immunogenicity of AZD1222, a non-replicating ChAdOx1 Vector Vaccine, for the Prevention of COVID-19" of AstraZeneca in USA, 30,000 participants have been randomized in a 2:1 ratio to receive 2 IM doses of either 5 × 1010 vp adenovirus vector based AZD1222 vaccine (n = approximately 20,000) or saline placebo (approximately 10 000) administered 4 weeks apart. The trial began enrolling in August 2020 and will complete enrolment by December 2020. It is expected to be completed by October 2022[36].

Following a vaccine recipient developing symptoms consistent with transverse myelitis, this trial was paused and later began enrolment in the UK and USA. One of the potential limitations of this vaccine is the need for refrigeration in ultra-low temperatures and this creates logistic problems in low-income countries[33].

Moderna mRNA-1273

A trial being jointly conducted by Moderna and National Institute of Allergy and Infectious Diseases, NIH, USA entitled "A Phase 3, Randomized, Stratified, Observer-Blind, Placebo-Controlled Study to Evaluate the Efficacy, Safety, and Immunogenicity of mRNA-1273 SARS-CoV-2 Vaccine in Adults Aged 18 Years and Older" is testing a mRNA based COVID vaccine[37]. It started enrolment in July 2020 and is expected to be completed by October 2022.

Of the 30,000 persons expected to be enrolled, half will receive one intramuscular (IM) injection of 100 microgram (μg) mRNA-1273 on Day 1 and on Day 29 and, the remaining half will receive a placebo, 0.9% sodium chloride (normal saline) injection[38]. This vaccine candidate, if it succeeds in phase III trials would also have a limitation for storage conditions of -20°C and is likely to limit its use in countries with limited resources and health infrastructure.

Pfizer and BioNtech mRNA Vaccine

Pfizer and BioNtech have also developed a series of mRNA-based COVID-19 vaccines. Because BNT162b2 showed a better breadth of T-cell responses and better safety profile, it was chosen for evaluation in phase 3 trials[33]. BNT162b2 requires storage at –80°C, a fact that could pose logistical problems. A phase 3 trial involving approximately 44,000 individuals (aged 18–85 years) is now taking place in the USA[38].This vaccine has received licensure in UK and is now being rolled out. However, its major limitation is the requirement of storage conditions of -80°C.

Johnson & Johnson's Adeno Virus Vector Vaccine

The Janssen Pharmaceutical Companies of Johnson & Johnson have initiated a randomised, double-blind, placebo-controlled, phase 3 trial on 60, 000 participants, aged 18 years and older of their replication-defective Ad26.COV2.S vaccine, which expresses full-length spike glycoprotein[39]. Results have been promising and a single intramuscular dose of the vaccine without adjuvant has been shown to result in a strong neutralising antibody response and provides protection against SARS-CoV-2 challenge in rhesus macaques[40].

Similar results are expected in humans. The vaccine candidate has an advantage that it requires storage at 2–8°C, which will be readily manageable in resource limited countries. Phase 1/2 trial involving 1045 participants (aged 18–55 years and ≥65 years) are ongoing in USA and Belgium, while the phase 3 trial of this vaccine already started on September 23, 2020.

Cansino Biologicals' Adeno Virus Vector Vaccine

CanSino sponsored Phase III trial of a COVID-19 vaccine of Adenovirus Vector in adults 18 years and above aims to enrol ~40,000 participants in Pakistan [recombinant novel Coronavirus vaccine, Adenovirus Type 5 Vector). The two-arm trial includes intramuscular administration in 20,000 participants and a similar placebo group of 20,000 participants. The trial is expected to begin enrolment in September 2020 and will complete enrolment by December 2020. It is expected to be completed by January 2022[41].

Concluding Remarks: *Where do we go from here?*

The quest for the most suitable platform as well as a safe, immunogenic and efficacious vaccine candidate is the current focus of global initiatives on COVID-19 vaccine development. Lack of translation of animal study results in human trials is a point of disappointment and identifying a vaccine that would elicit a strong cellular as well as humoral immune response is likely to be the main challenging goal for the vaccine manufacturers. Various vaccine platforms in clinical trials against SARS and MERS primarily used either the whole virion or *S* gene. However, in the case of COVID-19, a greater number of platforms are being tried using the same antigens. Available evidence indicates that adverse events associated with vaccine candidates using Spike protein or S1 is a concern; hence work on non-spike-based candidates will have to continue. Eventually, identification of more conserved proteins that are shared by all coronaviruses might provide a workable approach towards development of a generic coronavirus vaccine. Interestingly, majority of the ongoing clinical trials are not assessing mucosal administration of COVID-19 vaccine candidates. Because of their ease of administration, topically administered vaccines should be always kept as a development target.

Many COVID-19 vaccines are now fairly advanced in their clinical testing and some vaccine manufacturers have approached the country regulators for licensure and use before completing the recommended clinical Phase III evaluation in view of the global emergency and impact of the ongoing pandemic. Although we seem to have reached closer to the primary step of narrowing down on some potential vaccine candidates for COVID-19, it will be important to address issues such as large-scale manufacturing, establishment of supply chains and creating in-country capacities of vaccinating the targeted and prioritized populations. Although there is some movement towards arriving at a consensus regarding intensifying production wherever the capacity exists and ensuring vaccine distribution based on needs rather than financial abilities, actual implementation of such a decision might be full of hurdles.

Looking at the present sequence of events, the world seems to be poised to COVID-19 vaccine roll out by the end of 2020 or early next year. However, majority

of the ongoing clinical trials are expected to declare their results in 2021. Therefore, there will be some period of uncertainty wherein vaccinating the population will be implemented as per the respective in-country policies. But if the currently rolled out vaccine candidates fail in their Phase III evaluation; countries will find it difficult to cope with the challenged situation and alternative mitigation plans must be kept ready.

It might take several months to a year for a COVID-19 vaccine to be available for the masses after it proves to be safe and effective for human use and is granted licensure. Effective roll out and coverage would require a lot of ground work such as deciding priority population categories for vaccination, creating line listing of potential vaccine recipients, building stocks of the nationally approved vaccines and training staff that can be fully involved in vaccinations.

Once started, there should be in-country capacity to provide the required number of doses to populations prioritized for vaccine receipt. Engagement of developing countries' vaccine manufacturers' network members in research and development of COVID-19 vaccines as well as their capacities in manufacturing, fill-finish and vaccine distribution were assessed through online search, direct communication and an internal survey. It was concluded that the existing manufacturing, fill-finish and distribution capabilities of the network were optimum for roll-out of vaccines against COVID-19, while maintaining supply security of existing vaccines[42].

However, COVID-19 vaccination program will succeed only if there is adequate communication regarding vaccine safety, effectiveness, possible adverse reactions, follow-up care options and access to vaccines. It is critical to create awareness in all sections of the society through effective community engagement. The real challenge for the policy makers and program managers would be to balance public expectations against vaccine availability.

It will be important to boldly face the issues around vaccine hesitancy and barriers to vaccine uptake which will affect coverage and prove to be a problem in prevention and control of COVID-19[43]. Due to public demand, perceived public health need, interest among populations to receive vaccines, strong political will and interest of the Governments for vaccine roll out and interest among the vaccine manufacturers to position themselves as pioneers and global leaders in COVID-19 vaccine arena; countries have started receiving applications for Government level approval and licensure of the vaccines. In country regulators will have to take very learned decisions regarding this.

Vaccine roll out without a solid evidence of safety and efficacy might back fire due to serious and unexpected side effects even if they are rare and might harm the program because of the confusion and distrust that may be created in people's minds following such events[44]. Transparent and adequate communication will be the key for vaccine acceptance and uptake.

There is already a global debate on the lesser economies remaining deprived of adequate vaccine supply for their people. It would need global level understanding and consensus and this COVID-19 pandemic has provided an opportunity to develop models, approaches and systems that would benefit all and that too equitably.

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CHAPTER 7

COVID-19 INFECTION AND VACCINES: INDIAN RESPONSE

This chapter provides a brief about the current epidemiology of COVID-19 infection and disease, country's health system response to the pandemic and the vaccine pipeline.

District-wise COVID-19 Burden in India

Recent district wise estimates are represented in **Fig. 1 and 2** for understanding the national COVID-19 burden since March 2020 and the spread of the epidemic over time.

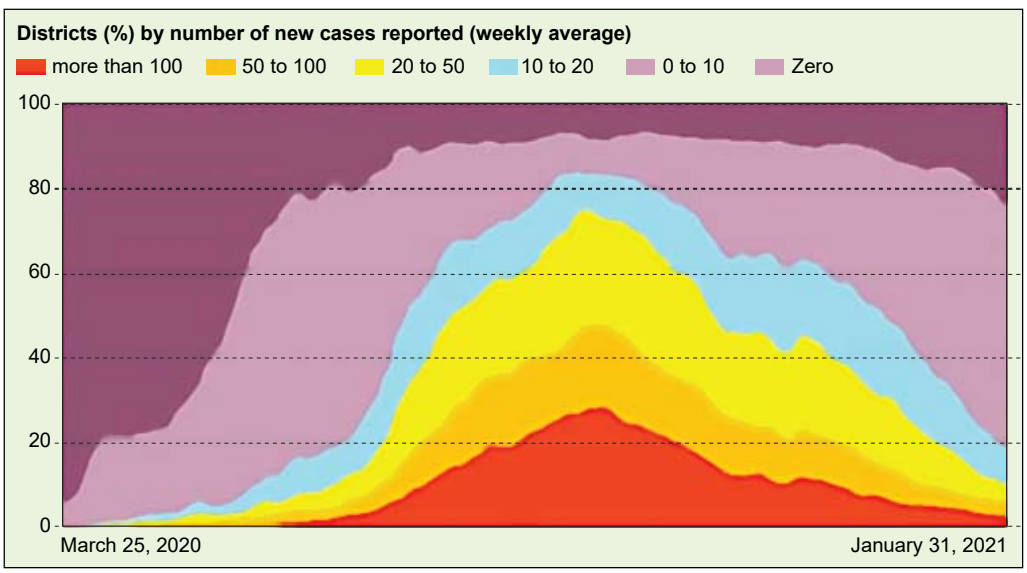

Fig. 1: *Number of new cases reported (weekly average) in analyzed 707 districts (%) (Source: HT Covid-19 dashboard (Hindustan Times February 10, 2021).*

The key take away from **Fig. 1** are: i) the maximum impact of the pandemic was only in 25% of the 700 plus districts of the country and ii) weekly average was nil in another 25% districts and most of the cases occurred in only 21 districts (3%). Clearly the impact of the pandemic was in a limited part of the country, mostly densely populated urban settlements. India reached its daily peak in mid-September and since then cases gradually declined till 31st January 2021 (at the time of writing of this chapter). Simultaneously, three rounds of sero-surveys in 70 districts were conducted: June 2020, September 2020 and January 2021. The sero-positivity increased from 0.7% to 7% to 23% through the three rounds respectively. This data again demonstrated that majority of the Indians are vulnerable and susceptible to COVID-19 infection and require constant vigil as well action by the stakeholders concerned. Sharp spike in the cases of COVID-19 in Kerala and Maharashtra after initial respite were testimony of the principle of maintaining constant vigil and impact of any complacency in the system and the community can be expensive.

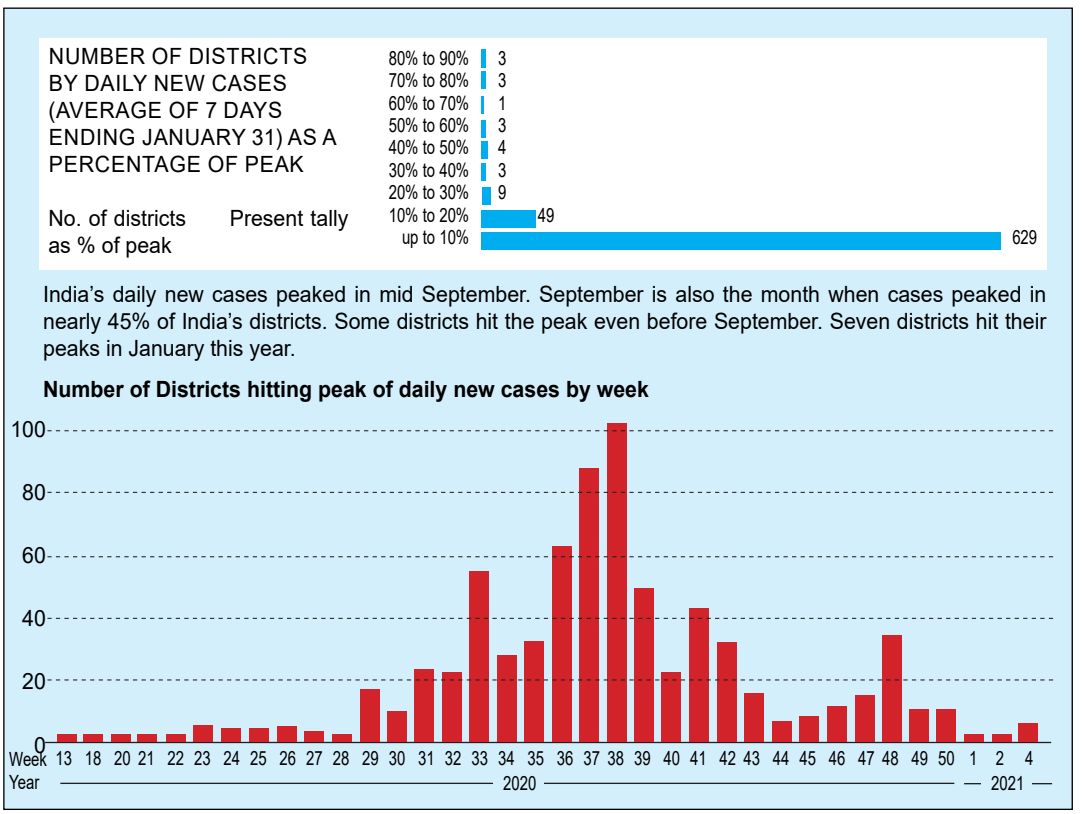

Fig. 2: *Number of districts hitting peak of daily new cases by week.*
(Source: HT Covid-19 dashboard) (Hindustan Times February 10, 2021)

Transformation of Indian health care infrastructure amid COVID-19 outbreak

The year 2020 will be remembered as a milestone for the Indian health system strengthening; the challenges of pandemic were transformed into an opportunity of filling some of the glaring gaps in the public sector facilities. Strict lockdown that India implemented in the first six weeks was exploited to expand the immediate requirements of diagnosis, quarantine, treatment and follow up of the COVID-19 patients. The progressive infrastructure development for COVID-19 treatment facility, isolation beds with and without oxygen support, ICU beds and ventilator bed is summarized in **Table 1 and Fig. 3**. Notably 5-10x time expansion occurred in a short period of 9 months.

Table 1: Infrastructure status for managing COVID-19 patients. (Source: Press information bureau, December 30, 2020)[1]

S.No.	Infrastructure	Numbers
1	COVID Treatment Facility	1,53,78
2	Isolation beds without oxygen support	1,267,127
3	Isolation beds with oxygen support	2,70,710
4	ICU beds	8,11,13
5	Ventilator Beds	40,627

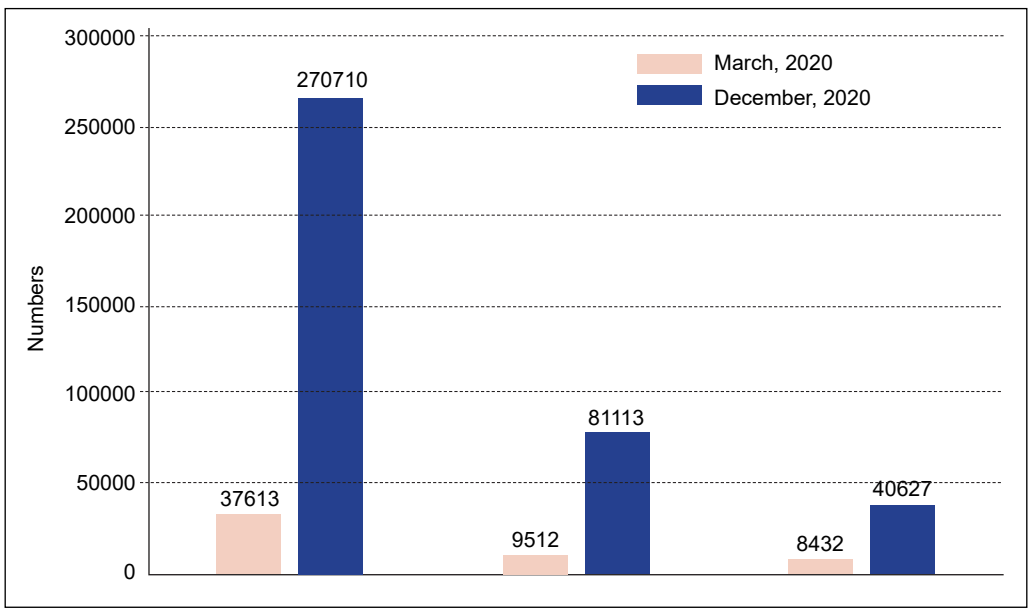

Fig. 3: *Transformation of Indian health care infrastructure amid COVID-19 outbreak of 2020 (Changes between March 2020 and December 2020). (Source: Ministry of Health and Family Welfare, Government of India)[2].*

The infrastructure development included several other domains viz. developing quarantine facilities **(Table 2)**, establishment of manufacturing facilities for personal protective kits (from zero to >4.5 lakhs/day), N-95 masks (from zero to >32 lakhs/day), and India emerged as leading supplier of anti-COVID-19 drugs (Ramdesivir, Hydroxychloroquine etc.). India might soon become the major supplier of COVID-19 vaccines to world besides fulfilling its own requirements. Another interesting and novel innovation was the conversion of village primary schools and Panchayat bhavans in to quarantine places for the migrant labourers and other visitors to the villages. If the infection had spread widely in the rural India where 70% of the population resides, the country could have observed unimaginable morbidities and mortality due to pandemic.

Table 2: Quarantine centres established/ converted in India. Distribution is shown for select states. A total of 879 quarantine centres registered across the country. (Source: Mission Vande Bharat, Government of India)

State	Quarantine Facilities
Andhra Pradesh	73
Bihar	30
Delhi	52
NCR	26
Goa	30
Gujarat	43
Haryana	76
Jharkhand	17
Rajasthan	111
Uttar Pradesh	30
West Bengal	60
Karnataka	113
Kerala	18
Madhya Pradesh	30
Maharashtra	52
Orissa	18

Exponential expansion of the molecular diagnostic laboratory network for COVID-19 occurred across the country; most of the districts have now access to state-of-art diagnostic facilities **(Table 3).** The private sector played an important role in this laboratory expansion exercise. Till February 2021, a total 2376 laboratories have become functional, of which 80% had achieved NABL certification. From being a net importer, Indian entrepreneurs started manufacturing more than 15 lakh diagnostic kits per day. Efforts have also been made towards establishing 'mobile infectious disease laboratories' to reach some of the inaccessible areas. The extensive COVID-19

testing capacity led to a daily testing of 1-1.5 million individuals every day and as of 14th February, 2021 over 206 million COVID tests were done in the country.

Table 3: Details of laboratory services and COVID testing laboratories in India. (Source: ICMR and NABL, February 10, 2021)[3-5]

S.No	Laboratory Services	Government	Private	Total
1	ICMR COVID Laboratories (For any test)	1217	1159	2376
2	Real Time RT PCR	541	815	1356
3	True NAT	632	254	886
4	CBNAAT	42	90	132
5	Other testing platform	02	00	02
6	NABL Accredited laboratory	1930		

With transformative changes in the infrastructure development and system strengthening over a short period of time, India was able to successfully confront the COVID-19 pandemic and the longitudinally maintained consistently low case fatality rate of ~1.4%.

COVID-19 Vaccine R&D and Candidate Vaccine Pipeline

India has emerged a major global player in vaccine manufacturing. Almost 2/3 requirement of the global vaccine needs are manufactured in India. Building on the existing industrial infrastructure, support of the Government of India through its science ministries and encouragement of academia-industry partnership, the country successfully built a healthy COVID-19 vaccine pipeline during 2020. In December 2020, under Mission COVID Suraksha, an impressive INR 900 crores was allocated for vaccine research and acceleration of the process of getting the vaccines into market.

On 16th January 2021, India launched the COVID vaccination program with two vaccines, both manufactured in India, one licensed from an international pharmaceutical company and another indigenously developed. Another important strategy for progress towards Atmanirbhar Bharat is to establish facilities for undertaking validated bioassays and small and large animal pre-clinical studies. A national network of 6-7 laboratories from both public and private sector is under preparation. International collaboration with CEPI which is the Coalition for Preparedness Innovations launched in 2017 to develop vaccines to stop future epidemics and other academic institutions will facilitate establishment of common protocols and external quality assurance mechanisms.

Globally, a number of different approaches have been used to develop a variety of COVID-19 vaccines. The DNA/RNA based COVID vaccines for human use are unique and used for the first timer. This will facilitate faster immunization of global populations to effectively fight the pandemic. As per most recent available updates, 63 candidate vaccines are in clinical stage globally, including 16 in Phase III, 6 in Phase

II/III, 5 in Phase 2, 18 in Phase I/II and 18 in Phase 1[6,7]. These clinical stage vaccines include various approaches and underlying technology including: protein subunit (20), viral vector (VV) (non-replicating) (10), inactivated virus (9), DNA (8), RNA(7), viral vector (VV) (replicating)(3), virus like particle (2), VVr+ antigen presenting cell (2), live attenuated virus (1) and VVnr+ antigen presenting cell (1).

Among the 16 vaccine candidates that are in Phase III globally, the vaccine technology platforms utilized include: inactivated virus (6), viral vector (non-replicating) (4), RNA (3), protein subunit (2), and DNA (1). (Please refer to chapter 6 for further insights on these vaccines in advanced stages of clinical trials and their recently initiated vaccination schedules).

Fig. 4 provides the status of 14 COVID vaccine candidates in advanced stage of development in India as of February 2021. There are at least three vaccines (Covishield, Sputnik V and Novavax) which have been developed and evaluated internationally and are also being evaluated in India as a part of the Phase III/bridging studies. Manufacturing facilities in India are at par with the global standards with affordable costs and hence all of the above mentioned vaccines are manufactured in the country. Johnson & Johnson vaccine was also trying to enter Indian market (at the time of writing the manuscript) through the same route – bridging Phase III trial and get the vaccine manufactured here in India.

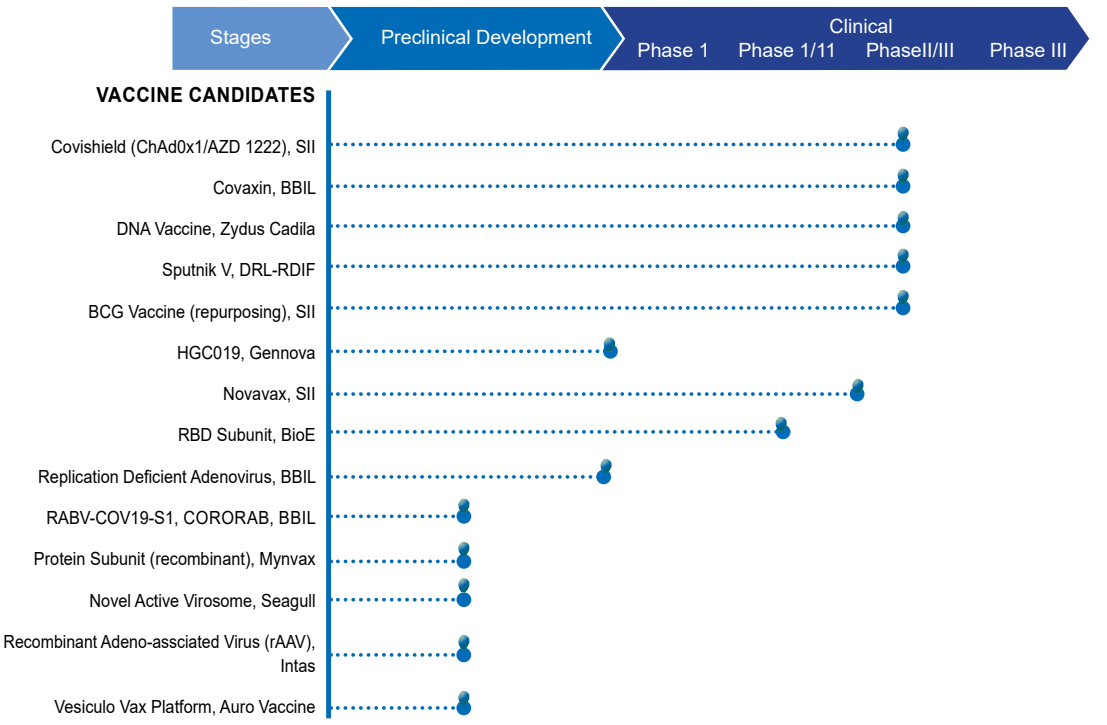

Fig 4: *India's COVID-19 vaccines in pipeline*[8].

In addition there are large number of vaccines developed on diverse vaccine platforms and later manufactured by Indian companies. This also includes the nucleotide-based vaccines (DNA and RNA vaccines). For the specific updated details, India's COVID-19 vaccines which are in clinical stage (Phase II/III) are summarized in **Table 4** along with their clinical trials status **(Table 5).**

Table 4: India's COVID-19 vaccine products which are in clinical stages[8,9]
(Acknowledgement: Jyoti Logani, Department of Biotechnology, Government of India).

Vaccine Candidate	Developed by	Clinical Phase	Vaccine Platform	Doses & route of administration	Storage Temp.	Updates
Covishield (ChAdOx 1/AZD 1222)	Serum Institute/ University of Oxford with AstraZeneca	2/3	Non replicating viral (modified chimpanzee adenovirus) vector	2 intramuscular doses. 4-6 weeks (upto 12 weeks.)	2-8 °C	Got emergency use approval on January 03, 2021. Expected to get WHO approval soon. Globally approved in >30 countries.
Covaxin	BHARAT Biotech with ICMR, National Institute of Virology	2/3	Inactivated whole virus	2 intramuscular doses at a gap of 4 weeks	2-8 °C	DCGI recommended emergency use based on phase 3 immunogenicity data for 24000 volunteers after 1st dose and 10,000 volunteers after 2nd dose. Vaccine showed good response against UK resistant variant.
Sputnik V	Dr Reddy's Laboratory with Gamelva/RDIF	2/3	Non replicating .....`` viral vectors (Ad-26 virus for the first dose and Ad-5 virus vector for the second dose)	2 intramuscular doses at a gap of 4 weeks	<18 °C 2-8 °C	Phase II completed in Jan, 2021, safety and immunogenicity data submitted to DCGI for 100 subjects. Phase 3 started.
ZyCoV-D	Zydus Cadila	2/3	DNA vaccine	3 intramuscular doses at a gap of 4 weeks and 8 weeks respectively	2-8 °C	Phase 3 initiated, EC approvals for 4 sites, 25 sites processing for EC, recruitment started at 2 sites. Pharmajet device required for immunization.
RBD subunit	Biological E with Baylor college of medicine	1/2	Recombinant Pichia pastoris platform	2 intramuscular doses	2-8 °C	Phase 1/2 approval by DGCI in November 2020. Non-human primate challenge study ongoing.
HGCO19	Gennova Biopharmaceu-ticals with HDT biotech corporation USA	1/2	mRNA	intramuscular	2-8 °C	Conditional permission by DCGI for phase 1/2 in December 2020. Plan to include 120 volunteers and conduct studies at KEM hospital Pune and Kolhapur government hospital.

Vaccine Candidate	Developed by	Clinical Phase	Vaccine Platform	Doses & route of administration	Storage Temp.	Updates
BCG Vaccine	Serum Institute	2/3	Genetically modified BCG vaccine	intradermal	2-8 °C	Recombinant BCG vaccine with M. Bovis urease C gene replaced by listerolysin O encoding gene hly. Data expected by February 2021. Repurposing for COVID-19.

Table 5: Clinical trials details and updates on various COVID-19 vaccines in advanced stages in India[8]. (Acknowledgement: Jyoti Logani, Department of Biotechnology, Government of India)

Vaccine	Trial sites	Sample Size	Age Group	Start Date	Key Details	Manufacturing Capacity
Covishield (ChAdOx1/ AZD 1222) Serum Institute of India	14	1600 (400 immuno-genecity cohort, 1200 safety	18-99	24/08/2020	Scientific evidence available[10-13]. Data from UK, Brazil, South Africa: Phase 3 interim analysis shows vaccine was safe, efficacy averaged 70.4% (62-90% depending on dose)	50 mn doses are ready, 500 mn doses by July 2021.
Covaxin Bharat Biotech	26	25800	18-99	11/11/2020	Phase 2 study shows tolerable safety outcomes with enhanced humoral and cell mediated immune responses[14]	150 mn doses annually.
Sputnik V, Dr Reddy's Laboratory	24 sites	1600 (100-Phase 2, 1500-Phase 3	18-99	21-01-2021	Interim analysis of the phase 3 trial of Gam-COVID-Vac (Sputnik) showed 91·6% efficacy against COVID-19 and was well tolerated in a large cohort[15,16]	Collaborating with Panacea:100mn doses/ annum Hetero 40-50 mn doses/ annum Virchow 40-50 mn doses/annum.
ZyCoV-D Zydus Cadila	60	28216	12-60+	15/01/2021	This is Nation's first indigenously developed DNA vaccine candidate against COVID-19. It has been approved by Drugs Controller General of India (DCGI), for conduct of the Phase III clinical trials[17].	6-8 mn doses/month and to be increased to 100-150 mn doses by March-April 2021.
RBD Subunit Biological E	5	360	18-65	16/11/2020	Phase 1/2 interim data on safety and immunogenicity is expected soon[18].	Ag capacity 1.5 bn/yr Formulation capacity: 1 bn doses/year.
HGCO19 Gennova Bio-pharmaceu-ticals		Phase 1: 120 Phase 2: 500	18-99		This is Nation's 1st indigenously developed mRNA vaccine candidate against COVID-19. Safe, immunogenic with neutralizing antibody activity in the rodent and non-human primate models[19].	40-60 mn doses/month by April 2021 Increase to 100-120 mn doses by 2022,

Vaccine	Trial sites	Sample Size	Age Group	Start Date	Key Details	Manufacturing Capacity
BCG Vaccine Serum Institute of India (VPM1002)	39	5946	18-99	21-04-2020	Evaluation of laboratory confirmed SARS-CoV2 infection among HCWs/ high risk groups/ with severe and critical life threatening disease[20]	Repurposing strategy.

There are several COVID-19 vaccine candidates in the pre-clinical stage of development and are expected to be available in the market in next 12 months. **Table 6.**

Table 6: India's COVID-19 vaccine candidates that are in pre-clinical stage[8]
(Acknowledgement: Jyoti Logani, Department of Biotechnology, Government of India)

Vaccine Candidate	Developed by	Vaccine Platform	Remarks
Vesiculo Vax	Aurobindo Pharma Ltd & Aurovaccine, USA	Replicating viral vector	Intramuscular route of administration. Storage at 2-8ºC. Vaccine based on live attenuated vesicular stomatitis virus (VSV) vaccine vectors. S protein gene was cloned into different VesiculoVax vector genomes.
Recombinant adeno associated virus (rAAV)	Intas Pharmaceuticals Ltd	Replicating viral vector	Replicating vectors produce new viral particles in the cell which infect other cells to enhance antigen expression and immunogenicity.
Novel active virosome	Seagull BioSolutions Pvt Ltd	Subunit	Active virosome platform.
Replication deficient Adenovirus	BHARAT Biotech/ Washington University	Adenovirus platform	Single dose intranasal vaccine. Studies in mice, hamsters and non-human primates reported viral clearance in the upper and lower airways and prevented symptomatic disease. Internal quality control is passed and is being assessed in CDL, Kasauli. Phase 1 approval is expected in February 2021.
Protein subunit (recombinant) vaccine	Mynvax	Protein subunit	Well established platform. 37ºC for a month storage. Proteins produced by microbial, baculoviral or mammalian technologies based on the need.
Codon deoptimized live attenuated vaccines	Indian Immunologicals/ Griffith University	Live attenuated using codon deoptimization technology	Storage at 2-8ºC. Uses the latest codon deoptimization technology which compromise viral replicative fitness making it harmless thus enhanced safety expected.
RABV-COV19-S1 CORORAB	BHARAT Biotech	Inactivated rabies vector platform	Rhabdovirus vector is well tested for human vaccines. Chemically inactivated RABV vaccines are safe and being used for humans. High expression of target antigens.

What is Expected from COVID-19 Vaccines?

Over the years, there has been considerable improvements in the field of vaccinology which resulted in consistent decrease in the time taken for vaccine development. **Fig. 5** summarizes timelines for development of some key vaccines against important viruses. Intriguingly, vaccine for Mumps was developed in the shortest time of 4 years as against the average time of approximately 10.7 years. To this end, the progress made in the direction of COVID-19 has truly been unprecedented with several vaccine candidates in advanced stages of development and already approved for emergency use within less than one year of the beginning of the pandemic.

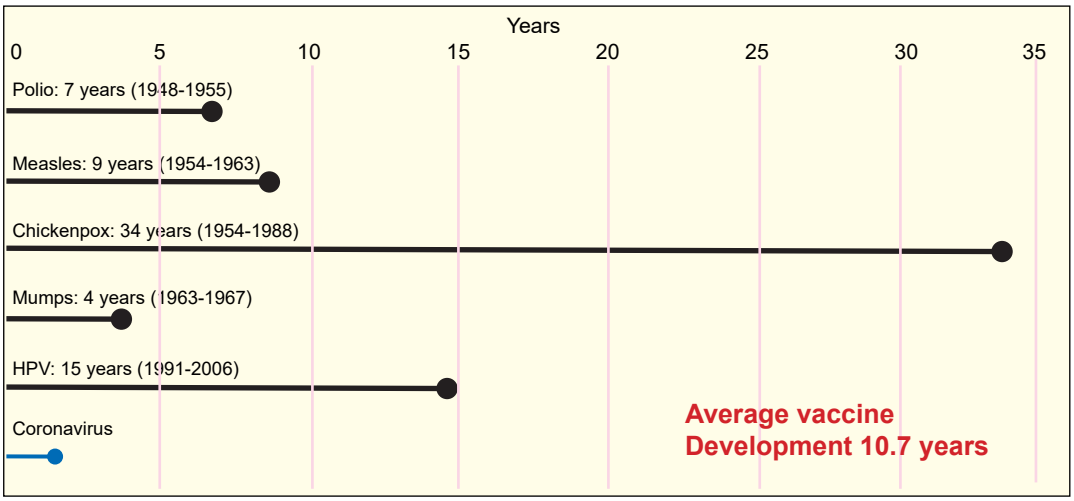

Fig. 5: *Vaccine development timelines for the vaccines against common viral infections.*

The goal of all these vaccine strategies is to stimulate a primary immune response and generate robust memory B and T-cells against SARS-CoV2 immunodominant conserved epitopes which should generate a long-lasting protective immunity against any subsequent COVID-19 infection. Based on the number of times the body is exposed either to the virus or vaccinated, broadly two types of responses are generated: *Primary immune response* on exposure to SARS-CoV2 for the first time or 1st dose of vaccine, which is slow and weak as it takes days for the body to generate appropriate T-cells and antibodies to eliminate the virus. However, this primary exposure should generate memory B and T-cells which will result in a stronger and quicker *secondary immune response* when exposed to virus again or to the 2nd dose of the vaccine, thus reducing the symptoms and severity through strengthened memory response **(Fig. 6).**

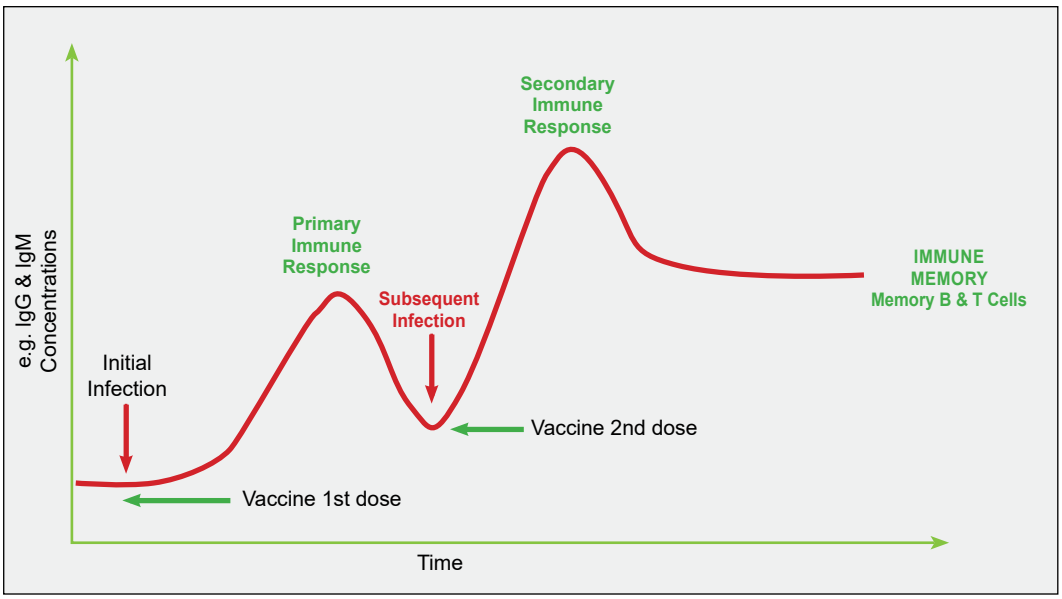

Fig. 6: *The principle of vaccinology as result of immune memory. Exposure to SARS-CoV2 for the first time or 1st dose of vaccine results in primary immune response (slow and weak) which generate memory B and T-cells leading to a stronger and quicker secondary immune response when subsequently exposed to virus again or to the 2nd dose of the vaccine which results in reduced symptoms and severity.*

Conclusions

India has established itself as the vaccine-manufacturing hub supplying almost 2/3rd of the global vaccine doses. The manufacturing infrastructure and active support from the state catalyzed vaccine R&D during the COVID pandemic. This has led to happy situation wherein i) India has introduced locally manufactured two COVID-19 vaccines in to the national program from 16th January 2021 onwards; ii) the country has committed a major contribution of vaccines to the global COVAX facility; iii) by the end February 2021, shared almost 20 million doses with 18 countries as bilateral arrangements; iv) the unprecedented strengthening of the health system scenario within a short period of 10-12 months; v) building on the existing immunization infrastructure and building a *de novo* IT platform for roll out of the vaccines; vi) focus on ATMANIRBHAR BHARAT along with financial support from state and academic partnership has released the enormous entrepreneur energy of Indians and several of the critical items required for diagnosis and management of COVID-19 are now manufactured in the country. It is a matter of pride for us that the country actually transformed from a net importer of several medical supplies and equipment to a highly valuable exporter; and vii) political leadership has realized the immense importance of investing liberally in the health sector as a step towards enhancing the economy of the country and international diplomacy.

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CHAPTER 8

ETHICAL ISSUES AND REGULATORY CHALLENGES

This chapter has two sections. The first discusses ethics of developing a COVID-19 vaccine and the second deals primarily with ethical issues after a vaccine is developed.

PART 1: Ethics of Developing a COVID-19 Vaccine

Vaccines have proved themselves to be effective in preventing diseases which once caused great public health concerns. The eradication of smallpox and the progress made with regards to polio reduction are classical examples of this. Routinely, modern scientific standards require vaccines to undergo years of trial and testing. The reason it takes so long is because of the numerous steps involved in ensuring that the vaccine is efficacious and safe.[1]

Typical Trial

A typical vaccine trial involves four phases of human studies. Before these phases, there are studies conducted on various animal models to test the safety and effectiveness of the vaccine. This is referred to as '*basic research or preclinical research*' which usually takes a couple of years. The phase one trial which follows establishes the safety and side effects (if any) of the vaccine by performing tests in healthy volunteers. The phase two tests the immunogenicity and safety of the vaccine in the trial population of interest. Phase three is where the efficacy of the vaccine is established across a population of wider demographics usually by comparing it with another vaccine, or a placebo vaccine. The number of trial participants goes up as the drug gets advanced through these phases. These three phases in total can take several years. Each of these phases would be accompanied by registering the trial and sharing the results with

the regulatory authorities of the country. After passing the three phases, the drug gets regulatory approval to be manufactured for the population. In Phase IV, the long-term effect/effectiveness of the drug and serious adverse events are monitored by the regulatory authorities. This phase can continue for a decade or more.[1]

A Vaccine for COVID-19

COVID-19 has brought an unprecedented challenge to global health systems. It has affected jobs and the economy, taken many lives, and has adversely impacted the health infrastructures. The advocacy on preventive measures like physical distancing, wearing masks and hygiene (frequent hand washing) has been useful but not concrete enough to prevent the rapid spread of the virus. As such, there is a belief that vaccines would be a key to the response against SARS-CoV-2.

This has led to rising demand and competition for a vaccine to be discovered in a short period of time. What usually takes many years to develop is now expected to be developed within the short span of a few months.[2] We have indeed seen this kind of accelerated vaccine development timelines being met with regards to some COVID-19 vaccine candidates already.

Careful mitigation of the ethical issues with regards to the participant recruitment, informed consent process, and distribution of the vaccine along with appropriate execution of duties and responsibilities of researchers, sponsors, and ethics committees are all essential to vaccine trials. However, with COVID-19 vaccine development, the time frame has been reduced to months; hence the ethical issues are much more pressing and require thorough and swift handling.

1. **Recruitment of participants:** Who should be recruited?

- Healthy volunteers (for P-I)
- Individuals at high risk (P-II, P-III)
- Individuals capable of consent (competent)
- Recruit vulnerable participants for inclusivity but with due safeguards from correctional facilities[3], and Pregnant/lactating women[4]

Ethics of recruitment: There are several ethical issues involved in the recruitment process other than selecting the right candidate. First, there should not be an element of coercion in the recruitment process *(principle of non-exploitation).* Second, the recruitment must be completely voluntary *(principle of respect for autonomy)* and third, there must be the inclusion of a diverse population (including the vulnerable groups) so that the benefits of participation are equally distributed *(principle of justice).*

2. **Informed Consent Process (ICP)**

Informed consent is a continuous process involving three main components – i) providing relevant information to potential participants ensuring competence of the individual, ii) ensuring that the information is easily comprehended by the

participants and iii) assuring voluntariness of participation.[5] This has to be combined with adequate documentation of the consent process. ICP is one of the principal requirements for the ethical conduct of research.

Disclosure: It refers to how much information is to be disclosed, how it is disclosed, and the setting in which it is disclosed. With regards to COVID-19, we might not yet adequately know the risks involved in vaccine candidates (including long-term effects and trans-generational impacts). All of this information would require to be disclosed in a non-technical language to each potential participant. It may also be very important to consider 'who' is disclosing this information. If the person disclosing it happens to be a treating physician of the potential participant, then there is a chance of implied coercion as the doctor-patient relationship in India is often paternalistic in nature.

Comprehension: Comprehension refers to correct understanding of the information disclosed by the researchers to the participants. Several factors impact the ability to comprehend. This includes age of the participants, their ability to read, cognition, their relationship with the researcher, and their preconceived ideas about research and vaccine. In particular, certain terms may be difficult to understand by the participant and will need to be simplified using relevant local examples. For example, concepts like placebo, randomization, and blinding.

Comprehension can be boosted by the use of multimedia (videotapes, audiotapes, interactive devices), enhanced consent forms (with well-adapted style, format, length, and language), use of tests or quizzes to assess understanding of the participant, and having extended discussions with the participant to communicate and solve doubts, concerns, and queries.[6] Primarily all written content such as the information sheet and consent form must use an accessible language without the use of medical jargon. In case it is necessary to use technical terms, they must come with a simple explanation.

Voluntariness: Some factors may affect the extent of voluntariness of the participant. In the Indian setting, there are different sets of power structures that may compromise participants' autonomy. In most clinical settings, there is a distinct power difference between the doctor (who can be also the person administering the consent), and the patient.

To mitigate such coercion, it must be ensured that the potential participants have enough time to read the information sheet, consent forms and to discuss them with their family or other key contacts, doctor as well as any other person they would like to reach out to. Secondly, participants must be encouraged to ask questions which must be addressed with honesty and transparency. Thirdly, ensuring the withdrawal rights of participants is paramount.

3. Transparency and accountability

It implies intentional openness, communication, and accountability operating in such a way that it is easy for others to see what actions are performed.[5] Transparency is important as it adds social value to the study and improves trust between all parties involved. It is also important in disclosing the planning process, research outcomes, potential and actual conflicts of interest and its management, stored data, sources of funding amongst others. Besides, there must be transparency regarding measures taken to ensure privacy and confidentiality of the participants. Any protocol deviation/ violation must be immediately disclosed to the institutional ethics committee.

Transparency should be prioritized by all stakeholders. This includes researchers, ethics committees, institutions, sponsors, regulators, policymakers, government, editors & publishers.

Ways to boosts transparency: Several measures can be taken to ensure transparency. Firstly, all clinical trials must be registered. In India, this is done online on the Clinical Trials Registry of India portal (http://ctri.nic.in/Clinicaltrials/login.php). Results must be voluntarily made available in the public domain through peer reviewed publications, reports, conference presentations and media outputs. All records about the study must be retained for a certain period (usually determined by the institutional ethics committee as per applicable regulatory norms). Also, there must be legitimate reassurance and public accountability to ensure trust.[7]

4. Reimbursement

It refers to determining and providing appropriate reimbursement for participants without causing an undue inducement.[5] This includes reimbursement for travel, inconvenience caused, other incidental expenses, indirect costs (such as loss of wages), and for the time spent. Reimbursements may either be monetary or non-monetary (educational support or medical benefits like insurance). Reimbursement rates in clinical trials, including for vaccines, need to be approved by the institutional ethics committee. Highlighting reimbursement being offered in clinical trials should not be ideally used as a way of recruitment.

5. Post-trial access or PTA

It refers to the supply of the investigational vaccine or its comparator to the study population and their communities and to determine if the vaccine is proven to be safe and efficacious. All participants in the control group must have free access to the vaccine candidate, ideally through a prioritized mechanism at the conclusion of the study. In addition, this facility might be extended to communities from which these participants are drawn, and which contributed to the vaccine development process.

6. Institutional arrangement/site selection. This includes the following:

Determining appropriate study centres that have apt resources

- Adequately trained investigators with suitable education and experience

- Trained support staff
- Medical resources including availability of facilities to transfer participants in case of a medical emergency[5]
- Adequate financial resources
- Supportive administration and leadership
- Training in Good Clinical Practices and Ethics.

7. Privacy & Confidentiality

Privacy is the right of an individual to control or influence the information that can be collected and stored whereas Confidentiality is the obligation of the researcher/research team/organization to the participant to safeguard the entrusted information.[5] *Ensuring privacy and confidentiality during vaccine trials is crucial to ensure that research participants are willing to participate. It is equally important to respect their rights.*

Ways to uphold privacy and confidentiality: The measures and precautions used to uphold a participant's privacy throughout the trial must be clearly explained in the ICP. The ways in which participants' health will be monitored must be disclosed. How long the data of the patient will be stored will also need to be informed. All identifiable information about the participant must be anonymized as far as possible in the research outputs.

Limitations in privacy and confidentiality must also be explained to participants. For example, it must be explained that it may not be possible to uphold confidentiality in the event of a court order. Researchers should also be well prepared regarding how they will handle incidental findings e.g. the discovery of a co-morbidity during the clinical work-up of a research participant.

Consequences of a breach: This is particularly important in the current context where there is much stigma associated with COVID-19. Further, a breach of confidentiality can result in a loss of trust leading to difficulty in retaining research participants in clinical trials, as well as can impact future studies. It could also contribute to vaccine hesitancy.

8. Ancillary care

If before or during the course of the study, a participant develops any disease which is unrelated to what is being studied, then provisions should be made for appropriate care. This may include care by the research team itself or referral to an external expert.

9. Role of Institutional Ethics Committees (IECs)

Institutional Ethics committees (IECs) play an important role in ensuring the ethical conduct of COVID-19 vaccine trials. Besides vetting the research proposal, including the information sheet and the consent form, IECs must also monitor the trial for any serious adverse events. In particular, IECs must monitor to ensure that there is no coercion or inducement, especially in situations where a high recruitment rate

is reported. It is also the duty of IECs to review the process of recruitment and to assess whether the inclusion and exclusion criteria are justified. There must also be involvement of lay members of the IEC in the review process. Finally, in the light of necessity to expedite the review process without compromising the quality of review, and given the importance of risk minimization at times like during an infectious pandemic, it may be necessary to come up with new ways of conducting the review such as the use of software (and other ways) to promote virtual review as recommended by WHO in Guidance for research ethics committees for rapid review of research during public health emergencies.[8]

10. Role of regulation

There have been concerns regarding regulatory authorities prioritizing COVID-19 vaccine trials by ignoring other important clinical trials. While this may be justified to an extent, the risks must not outweigh the benefits of doing so. Further, when it comes to multi-country trials, it is necessary to harmonize regulatory policies and pathways across nations involved in a particular trial.[9]

11. Controlled Human Infection Model (CHIM) studies

Controlled Human Infection Models or human challenge studies have been advocated as a potential way to help expedite the development of COVID-19 vaccine candidates. Human challenge study is an established model which purposefully infects humans with an infectious agent in a controlled situation to achieve relevant and generalizable endpoints of infection or disease. It helps in calculating a reproducible attack rate.[10]

Significance of approach: The CHIM platform could help in developing vaccine candidates quickly, with fewer participants and help in fast tracking. However, concerns around technical issues such as microbial factors (strain, inoculation method)[11], need for defining the end points of interest, and generalizability of results needs adequate attention.

There are general and unique ethical issues with regards to the use of CHIM approach.

General ethical issues: These include the scientific validity and feasibility perspective of the approach from the public health point of view. Running CHIM studies requires a lot of groundwork and specialized infrastructure which might take time to develop. It is also crucial to focus on community engagement, ensure attention to site, participant selection, as well as independent review and oversight.

Unique ethical issues: Specific ethical issues include the fact that there is a deliberate harm caused to the participants who are healthy persons (which could be considered a violation of non-maleficence). There might be no direct benefit to the participants, and no true 'right to withdrawal' from the study. The risks and benefits involved will need adequate explication. Power hierarchies involved in rich countries sponsoring

CHIM studies in low-resource countries will also need to be considered.[11]

Addressing ethical concerns: Sponsors and investigators will need to spend adequate time on doing quality community engagement, ensuring the right set of participants, sites and facilities [12] are chosen. It is also important to develop robust informed consent process, and appropriate mechanisms for reimbursement. If there are incentives paid to participants beyond reimbursement, including risk model payments,[13] the same would require close scrutiny.

The WHO in May 2020 released key criteria for the ethical acceptability of COVID-19 human challenge studies.[14]

- Scientific justification being sound.
- Risk-benefit analysis, with benefits outweighing risks.
- Systematic approach to consultation and engagement with the public as well as relevant experts and policy-makers.
- The studies should involve close coordination between researchers, funders, policy-makers and regulators.
- Site selection should ensure that the highest scientific, clinical and ethical standards can be met at the site(s).
- Participant selection criteria must focus on limiting and minimizing risks.
- Such studies should be reviewed by specialized expert committees which are independent decision makers.
- Rigorous quality informed consent requirements.

12. Emergency Use Authorization (EUA): Specific challenges

Globally, the need for rapid vaccine development for COVID-19 has led to approvals under special regulatory authorization mechanism called Emergency Use Authorization. These are based on interim data without waiting for full datasets of clinical studies (either bridging studies, or phase 3 efficacy studies). In India, such approvals have already been issued for two candidate vaccines under Restricted Emergency Use mode.

Ethical issues with regard to EUA approvals include the following:

- ***Scientific validity:*** Regulatory decisions taken in the absence of adequate long-term safety data, immunogenicity and efficacy (especially local) could raise concerns on validity for regulatory decisions. Balance between risks and benefits of EUA decisions must be adequately considered, and the rationale and details of such regulatory decisions must be put out in the public domain.
- Decisions using an EUA pathway should follow clear regulatory frameworks which allow for fast-tracking and approvals based on interim data and be reflected in legal guidance applicable in country. This should also be linked to clear oversight and monitoring mechanisms.
- Any applicable conflict of interest must be adequately recognized and managed in a transparent manner. These include ensuring recusal from decision making

of any individuals and bodies that might have a conflict of interest, and due care when there are partnerships between government bodies and private entities.

- The impact of such decisions on the ongoing trials for vaccines that have been given EUA needs due consideration, if these trials are to continue. It might be in scientific interest to maintain the trial design, but ethically it would be important to update the participants about the availability of the vaccine candidate under EUA mode, and this would require updating the informed consent. Participants will retain their rights to withdraw from the trial.
- EUA decisions will also impact future decisions around use of placebo arm for trials involving new COVID-19 vaccine candidates. Regulators, including expert committees providing approvals for clinical trials will need to examine what would be the norms regarding applicable 'standard of care' as this would define whether the vaccines approved under EUA can or should be considered the new standard of care. Alternatively, in the absence of full licensure and limited availability, it would not be considered the new standard of care. If the EUA vaccines are considered to constitute a new standard of care in-country, then future vaccine candidate trials may need to be run with the EUA vaccine as comparator, rather than placebo-controlled trials.

13. Community and public engagement

In the context of COVID-19, all citizens, especially those at high risk constitute the community. In discussing the community and public engagement in the context of COVID-19 vaccination, it is important to define who the stakeholders are.

Stakeholders: A pandemic subsumes much of the population across the globe directly or indirectly. All of the general public whether we regard them as citizens of the globe, individuals, or our fellow humans are important stakeholders. There may be a further categorization of vaccine users in general as those who have already been vaccinated and those who will soon be vaccinated, as well as the 'communities' which they belong to.

Following are those who are the primary duty bearers in combating the pandemic. This includes the medical doctors, clinical nurse, and other paramedics including health workers and health mediators. Countless registered (and unregistered) non-governmental and non-profit community based organization which engage in humanitarian, economical, support/relief centric work are also important stakeholders for their experience in working on the ground. Educational institutes from primary schools to universities and media and journalists are also key stakeholders for they have the power to inform and influence. This list of influencers can easily be expanded keeping in mind the role of the internet and social media.

Those in the government, political parties, bureaucrats and policy makers are also important because of their key role in decision-making. Here a series of ministries- education, health, information and broadcast, economics amongst others

are stakeholders in themselves. It is crucial to identify these and other stakeholders as they all play a role in the availability of information and transparency around COVID-19 vaccines. It is also important to delineate all relevant communities which need to be worked with.[12]

Approaches to community engagement: Several measures can be taken to ensure community engagement during COVID-19 trials. Communities have gatekeepers or people who are in a leadership position which can serve as a gateway to convincing a community regarding the benefits of vaccination. Religious leaders, especially for the religious minorities are one good example here. There were concerns with regards to polio vaccine in small pockets in India. And engagement with religious leadership helped a great deal in deal with these suspicions. Community members can be recruited as a part of the research team/health advocacy to help in understanding the sensitivities of the community. Focus group discussions and forming a community advisory board can be a very efficient strategy given the time restraint.[13]

Overarching principles of engagement: Throughout the process of engagement, certain overarching principles are to be always upheld. Inclusivity is one such principle where everyone is treated with respect and their inputs are valued. Inclusivity with regards to gender, age, educational and economic status must be upheld both within and between the communities. Disagreements if any should be settled peacefully without compromising the scientific validity of the trial. The process of engagement should not be perceived as just an activity but rather as an opportunity for mutual learning and strengthening health literacy.[14] Sharing of results including negative/ inconclusive results is therefore paramount.

Benefits of engagement: Good community engagement improves trust in the vaccine development process. It can make the conduct of research smoother and boost the chances of securing funds. It helps in faster dissemination of results and their uptake into policy. Community engagement can help in better handling of culturally sensitive issues and increase cooperation from participants. Further, it upholds the social value of the study, contributes to health research literacy and, minimizes power inequalities between researchers and participants.

14, Advocacy and communication

Health activism is the promotion of activities and initiatives which are aimed at improving the health of individuals, communities and the public at large. Advocacy is imperative in the development of the COVID-19 vaccine since "a pandemic is a communication emergency as much as a medical crisis".[15] The WHO, in fact, has used the term 'infodemic' which refers to the *state when the excessive amount of information becomes detrimental to addressing the issue*.[16]

Why is advocacy important? Advocacy is important to combat misinformation, false information, lack of trust, accusations, and conspiracy theories during the pandemic. It could also play a role in addressing the growing anti-vaccine movement.[17]

Strategies for advocacy: Key guidelines for developing a proactive COVID-19 pre-vaccination strategy includes the following:

- Behaviour change planning- Taking lessons from the past and following guidelines by credible institutions like WHO, CDC, and national bodies.
- *Audience targeting and segmentation*- Identifying groups of people with similar behaviour and attitude to plan advocacy strategy accordingly. Using quantitative and qualitative investigations in this regard.
- Competition and barrier analysis- Tackling false information or lack of information with accurate information. (Negotiating political differences).
- Mobilization- Coordinating with NGOs, private sector, religious institutions, trade unions for promotional and logistical assistance.
- Vaccine demand building- Using social media and health communicators.
- Community engagement.
- Working out vaccine access strategy- Risk-based approach i.e., those who are at higher risks (like health workers) will get the vaccine first.
- Using conventional, digital & social Media.[17]

PART 2: Ethical Issues After a Vaccine is Developed

1. Vaccine availability and positioning in the national context

Once a vaccine(s) with good safety and efficacy profile become available, the next question is whether or when there would be enough vaccines for reaching public health aims such as herd immunity. Currently there are several estimates. Let's look at a few.

- Airfinity, a life-sciences market analytics firm in London, currently projects that just one billion doses will be available by the fourth quarter of 2021.[18]
- CEPI based on anonymous surveys estimates 2-4 billion doses by end of 2021
- In India, the Serum Institute of India reported in October that limited doses would be available by Q2-Q3 of 2021.[19]

The quantity of doses required by health systems will also depend on the number of doses required per person to achieve herd immunity.[18]

Back in May 2020, the Washington post reported "If SARS-CoV-2 establishes itself as a stubborn, endemic virus akin to influenza, medical experts say, there almost certainly will not be enough vaccine for at least several years, even with the unprecedented effort to manufacture billions of doses."[20] While there have been several developments since this report, the possibility is real. In addition, timelines for availability of the vaccine would vary from country to country. This brings us to the next issue of looking at the ethics of how vaccines are distributed between countries and whether it is possible to ensure that there is a fair allocation of vaccines to India.

2. Vaccine distribution between countries: Ensuring India has enough vaccines for itself

Currently, there are no international agreements that govern equitable distribution of vaccines across the world. Fears of certain countries dominating vaccines in the early phases are real due to past experience with the 2009 H1N1 Swine Flu vaccine when wealthy countries allocated the bulk of vaccines to themselves at the cost of poorer countries. Nations where manufacturers are located have the possibility of limiting vaccines produced to their own citizens through the use of instruments such as mandatory export controls.

Equitable distribution between countries is important as COVID-19 cannot be contained within borders and it is important that herd immunity is achieved in all countries to prevent another global catastrophe. Further, the value of global equity, solidarity and a spirit of international cooperation necessitates that available vaccines are distributed based on need. Finally ensuring vaccine availability across the world is necessary in order to reactivate global supply chains which are vital to global economic recovery.[21]

Several instruments are being used to ensure vaccine sufficiency.

Option 1: *Use of Bilateral Advance Purchase Agreements (APAs)*

"APAs are legally binding contracts whereby one party, such as a government, commits to purchasing from a vaccine manufacturer a specific number or percentage of doses of a potential vaccine at a negotiated price if it is developed, licensed, and proceeds to manufacture."[22] They were used during the 2009 influenza A H1N1 pandemic as well but are a "gamble and erode collaboration between countries" and can increase inequities. But "some wealthy nations have secured more than 2 billion doses of potential future COVID-19 vaccines using APAs."[18]

Countries that have signed APAs include USA (800 mn doses), UK (340 mn doses, highest per capita), EU, Japan, Brazil and Indonesia.[18]

Option 2: *Multilateral agreements*

In light of the possibility of poorer countries being left behind in the race to be vaccinated, several international organisations have taken leadership in order to establish multilateral agreements to ensure equitable vaccine distribution between countries. The most notable among them is the Advance Market Commitments (AMC) used by the Gavi, The Vaccine Alliance. Gavi has used donor-funded AMCs to enter into APAs with vaccine manufacturers. This will ensure that a guaranteed number of vaccine doses are supplied to countries with limited profit-based markets. Such arrangements have been used in the past as well with childhood pneumococcal vaccines and Ebola vaccines.[22]

The arrangement made under the leadership of the GAVI is the COVID-19 Vaccine Global Access (COVAX Facility) AMC. It is co-led by GAVI, CEPI & WHO. Launched in April 2020, participating governments can access a portfolio of vaccine

candidates so that risk is minimised in case some of the vaccines are not efficacious enough or have a poor safety profile.

Another existing legally binding framework which has some relevance to COVID-19 vaccine allocation is the International Covenant on Economic, Social, and Cultural Rights (ICESCR) signed in 1966. Around 171 countries have legally binding obligations under it. For example, the covenant mandates countries "to take steps, individually and through international assistance, to realise the right to health and the right to enjoy the benefits of scientific research and its applications, without discrimination"[22]. However, like other UN mandates, the ability to enforce it is limited.

Option 3: Export Controls

Export controls are national level mechanisms used to limit domestic manufacturers from exporting COVID-19 vaccine to other countries and can be used to supersede existing APAs. While export controls go against the idea of free market and international cooperation, they have been used in the past, for example, during the 2009 influenza A H1N1 pandemic when high income countries used them to ensure vaccine availability to their own citizens until domestic needs were met.[22] Export controls also go against obligations under ICESCR 1966.[23]

Vaccine manufacturing nations such as India have the potential to use such methods; however this might be balanced against the need to show global leadership in sharing vaccines with other countries to cover especially priority/high risk populations, and its role as a health diplomacy platform.

Option 4: Compulsory Licencing (CL)

Compulsory Licencing works by forcing international vaccine developers to compulsorily licence the vaccine to domestic manufacturers. It thus allows domestic manufacturers to produce a vaccine without the consent of the patentee. Internationally, this possibility is recognised under the TRIPS agreement. However, compared to single molecule drugs, vaccines are more complicated for use of CL.[18]

3. Vaccine Allocation/Distribution within India

Why is vaccine allocation important?

In the last section we looked at the ethics of vaccine allocation between countries and how India can ensure that there is enough vaccine for the whole country. Nevertheless, at least in the initial stages, COVID-19 vaccines will be limited in supply and there will likely not be enough vaccines for all Indian residents. Moreover, to make efficient use of limited resources for vaccine distribution, it is imperative that there is a clear plan on how the vaccine will be allocated and who will be vaccinated in what order.

Concerns of equity are also important in vaccine allocation decisions. This is because equitable distribution is necessary to achieve herd immunity faster. If certain populations are systematically left behind in vaccine allocations, they could act as COVID-19 hotspots.

How can we decide on vaccine allocation?

Vaccine allocation is a major issue across the world. Nations have had active deliberations on deciding the priority groups while the scientific bodies have released frameworks for the equitable allocation of vaccines. This includes frameworks prior to the current pandemic and ones that were formulated after COVID-19 pandemic started. Some examples are as follows:

County	Body	Date of release	Framework
USA	Johns Hopkins University	August 2020	Johns Hopkins Interim Framework for COVID-19 Vaccine Allocation and Distribution in the United States [24]
Global	WHO	September 2020	WHO SAGE values framework for the allocation and prioritization of COVID-19 vaccination.[21]
USA	NASEM	October 2020	NASEM Framework for Equitable Allocation of COVID-19 Vaccine([25]

On September 14, 2020, the WHO released a document named the WHO SAGE values framework for allocation and prioritization of COVID-19 vaccination.[21] It identifies 6 core principles, 12 objectives and subsequently identifies different target/ priority groups. The Framework is designed to address ethical issues relating to the allocation and prioritization of COVID-19 vaccines.

The 6 core principles are as follows:

Principle	Description
Human Well-Being	Protect and promote human well-being including health, social and economic security, human rights and civil liberties, and child development.
Equal Respect	Recognize and treat all human beings as having equal moral status and their interests as deserving of equal moral consideration.
Global Equity	Ensure equity in vaccine access and benefit globally among people living in all countries, particularly those living in low-and middle-income countries.
National Equity	Ensure equity in vaccine access and benefit within countries for groups experiencing greater burdens from the COVID-19 pandemic.
Reciprocity	Honor obligations of reciprocity to those individuals and groups within countries who bear significant additional risks and burdens of COVID-19 response for the benefit of society.
Legitimacy	Make global decisions about vaccine allocation and national decisions about vaccine prioritization through transparent processes that are based on shared values, best available scientific evidence, and appropriate representation and input by affected parties.

In addition to the core principles, the Values Framework mentions that the following additional factors must be taken into account for vaccine allocation

- Information about specific characteristics of available vaccine or vaccines (e.g., age specific vaccine efficacy and safety).
- The benefit-risk assessment for different population sub-groups.

- The amount and pace of vaccine supply.
- The current state of epidemiology, clinical management, public health response, and economic and social impact of the pandemic.

Other complementary ways for equitable allocation

Prioritising by regions*:* It is possible to use vulnerability indices to prioritise certain districts over others. Acharya and Porwal have provided a vulnerability index in a recent paper.[26]

Prioritising based on seroprevalence*:* Seroprevalence surveys can be used in two ways. i) Districts or blocks which already have a high seroprevalence may be of lower priority. ii) Districts or blocks with a medium level seroprevalence can be prioritised for vaccination as these can be considered as 'low hanging fruits' where herd immunity can be achieved quicker.

4. Ethical issues with COVID-19 vaccination process

Making COVID-19 vaccines mandatory

World over the issue of liberty has come up during the pandemic in the context of mandating public health measures such as mask usage and stay-at-home orders. Similarly, in the context of vaccinations, the concept of liberty has been used by proponents of the vaccine hesitancy movement. Similar concerns may arise during the COVID-19 vaccination drives as well.

Mandatory vaccination requirements have been used in the past, and currently exist in several countries in the context of routine immunisations. For example, in several countries' insurance coverage or eligibility for school admission is linked to vaccination thus mandating vaccinations for children. Australia has a "No Jab, No Pay" scheme which withholds child benefits if a child is not vaccinated.

Similarly, COVID-19 vaccines could be mandated by governments, employers, education providers, residents' associations, etc. For example, employers may ask employees to be vaccinated before returning to work in the office setting. Similarly, schools may mandate pupils to be vaccinated before returning top schools.

Mandatory COVID-19 vaccination is being suggested as a solution to the prevalent vaccine hesitancy. Recent studies from UK and other countries show that 17% and 28% of people would refuse or hesitate to take the COVID-19 vaccine.[27,28]

Some authors have suggested that the only reason to mandate COVID-19 vaccination is on the grounds of the amount of risk a person who refuses to get vaccinated poses to others.[29] This is based on the argument of John Stuart Mill who states that the only ground for a state to use coercion is when an individual poses risk of harm to others. Savulescu[35] goes on to explain four conditions which must be met in order to make COVID-19 vaccination mandatory:

- There is a grave threat to public health.
- The vaccine is safe and effective.

- Mandatory vaccination has a superior cost/benefit profile compared with other alternatives.
- The level of coercion is proportionate.

However, making vaccines mandatory brings with it several issues which need to be thought through in advance. These include a) a nudge is considered better than a push as per behavioural theories, as it allows for behaviour change without necessarily impinging on liberties, b) the vaccine, especially launched under emergency authorization mode, may not be safe for everyone and c) it may further decrease trust.

Use of biometric IDs in vaccinations

The issue of liberty, and privacy, also come up if biometrics-based identifications, such as Aadhaar is made mandatory for vaccination. Even without an ID, biometrics such as fingerprints and iris scan itself can be used as an ID. Several persons are pushing for the use of a biometric ID to prevent duplication and to promote effective monitoring of COVID-19 vaccination drives. On the other hand, others bring up that past successes in India such as with Polio was achieved without the use of a biometric ID.

Advantages	Potential Problems
It can prevent double-dosing It can reduce exclusion Can help in better targeting Can help in tracking	- There is a risk that biometric based vaccination records may be used to create "immunity passports" with linked issues. - Biometric data may be hacked or leaked. - It may be sold to third parties. - Many people may not feel comfortable or safe giving biometric information.

If India plans to use biometrics or biometric-based IDs for COVID-19 vaccination, mechanisms must be put in place to not exclude those who may not have such IDs or do not consent to the use of their biometrics. Ensuring that there is a 'non-biometric' alternative way to get vaccinated will help respond to ethical and practical concerns.[30]

Use of Vaccine "Passports"

Vaccine passports are also linked to the idea of liberty. Also called as "immunity passport" or "immunity-based licences" or "risk-free certificate", vaccine passports are given to people who show evidence of immunity to COVID-19 on the basis of antibody testing or vaccination. However, correlation between presence of antibodies and immunity is unclear. In addition, even if immunity is present, its duration is unclear. In a scientific brief on 24 April 2020, WHO advised against the use of such immunity passports.[31] Further, there is a possibility of discrimination against those who do not have the passport.

However, others have argued that use of "immunity-based licences" has the potential to reduce inequality and reduce duration and extent of widespread public

health measures such as lockdowns and travel restrictions. Persad and Emanuel[32] argue that immunity-based licences offer several benefits such as economic benefit through targeted licensing for attending cultural, worship and sporting events which can boost the economy and can help in reducing infection transmission. However, the ability to make effective immunity passports depend on the validity and reliability of serology tests for antibodies and knowledge on the duration of immunity provided by prior infections and vaccinations.

5. Ethical issues associated with infrastructure provisioning for COVID-19 vaccination

Other than ensuring enough vaccines for India, and their equitable allocation, the process brings up several ethical challenges. The major challenge is that of human resource and vaccine infrastructure. Firstly, India suffers from an already weak public health infrastructure. Even before the pandemic, there were not enough beds, doctors, nurses and other healthcare workers.

Vaccine coverage for routine immunisation is poor in many states, a consequence of weak infrastructure. Now, COVID-19 places an unprecedented demand on the public health infrastructure. This is because routine vaccinations are limited to mothers and children. The system needs to be upgraded to vaccinate vast majority of the Indian population.

It has been estimated that for vaccinating a targeted 70 crore population over a period of six months, a pool of four lakh staff dedicated only to COVID-19 vaccination would be needed.[33] There have been numerous suggestions on how to get these required human resources. One suggestion is to use students of different health professions. India has roughly about 80,000 MBBS students, 26,000 BDS students, 55,000 nursing students and 30,000 AYUSH students annually in each cohort. However, using the student population has the risk of affecting their studies and training resulting in a future cohort of poorly trained health professionals.

Others have suggested redeploying existing healthcare human resources. But this brings several ethical issues with it. For example, using existing human resources for health has the risk of compromising routine immunisation and other routine public health activities for the sake of COVID-19 vaccines.

Another suggestion is to use an "election like arrangement". Whether this means that school teachers will be roped in and schools used for COVID-19 vaccination is not clear. In addition, elections last only a few days at a time. COVID-19 vaccination will take much longer, probably months. This means that for that much longer period, the schools might not be reopened thus negatively impacting education outcomes further. Also, vaccines for COVID-19 require trained vaccinators, and there might be risks with using others who are not health professionals for this purpose.

6. Adverse events and associated ethical issues

No drug or vaccine has 100% safety.[34] and there is always a risk of associated adverse events. An adverse event is defined as "any health problem that happens after a shot or other vaccine".[35] However, despite the risk of adverse events, vaccines are implemented in large scale immunization programs because of the relative benefit of preventing death and disability.

An effort to understand if a vaccine is safe starts even before the clinical trials, through the clinical trial period and through post trial safety monitoring and surveillance. Before clinical trials extensive lab-based testing is done to check for safety. Through human trials, understanding the safety profile is of key interest and a vaccine is given approval only if found to be reasonably safe, effective and its benefits outweigh risks. It is important that regulatory oversight is stringent to keep track of vaccine safety concerns, and to ensure that information on vaccine safety is available transparently to all relevant stakeholders.

Serious Adverse Events (SAEs) which happen during vaccine trials should be promptly catalogued, reported as per applicable regulatory and ethics standards, and causality assessment completed in a timely manner. Furthermore, information on this assessment and its conclusion should be made available to the impacted participant/ their family, and also publicly communicated in due course.

Adverse events after vaccine roll out: Many adverse may be picked up only once Phase 3 clinical trials are complete, and the vaccine is being rolled out. This is because some adverse events may be too rare to detect in the smaller samples of Phase 3 trials. Secondly, some adverse events may be delayed and, hence, may not be possible to detect within the short window of trials. This is, especially, important in the context of the accelerated timelines for the COVID-19 vaccine trails. Finally, some adverse events that occur only in certain subpopulations may not be detected during trials if they have been excluded or inadequately powered in the trials.[36,37]

Surveillance: In order to detect adverse events after vaccine roll out, it is important to establish surveillance mechanisms. Surveillance is the systematic process of monitoring the recipient population for possible vaccine related adverse events. This is important as several adverse events may not be picked up in the clinical trials. This information should be collected systematically, analyzed and used to inform any updates in vaccination strategy on a real time basis.

7. Vaccine Hesitancy

At a larger level, surveillance is necessary as confidence in the importance, safety, and effectiveness of vaccines has fallen in many countries[38]. Adverse events, which may or may not be related to the vaccine have the potential to undermine the entire vaccination effort and can increase fear of the pandemic among the public.[36] There are numerous examples in the past where concerns about safety and necessity of vaccination have led to a drop in immunization coverage resulting in a resurgence

of disease.[37] Finally, surveillance is important in the context of the growing vaccine hesitancy movement. In 2019, WHO declared vaccine hesitancy as one of the top ten global health threats.

Types of adverse events[36]

- Adverse events of special interest (**AESI**)
- Adverse events following immunisation (**AEFI**)
- Other coincidental events that may be attributed to the vaccine

AESIs are "events of significant medical and scientific concern specific to the sponsor's program or product."[36] One possible AESI which can significantly affect vaccination efforts is the possibility of a vaccine-enhanced disease. This is when vaccination could make subsequent infection with SARS-CoV-2 even more severe. This had occurred previously with Dengue vaccine and had been reported with formalin-inactivated respiratory syncytial virus (RSV).

Other possible AESIs potentially include respiratory (including pneumonia, acute respiratory distress syndrome), cardiac (including cardiogenic shock, cardiomyopathy, arrhythmia, coronary artery disease, myocarditis and pericarditis), acute renal, and hepatic injury, neurological (including encephalopathy, encephalitis, GBS, anosmia and ageusia), sepsis and septic shock, hypercoagulability, rhabdomyolysis and multisystem inflammatory syndrome in children.[36]

Adverse events following immunisation (AEFIs) are "any untoward medical occurrence which follows immunization and which do not necessarily have a causal relationship with the vaccine usage."[36] Other than AESIs and AEFIs, surveillance is important to detect other coincidental events that may end up being attributed to the vaccine (for example: Autism following MMR vaccine, SIDS following whole cell pertussis vaccine.[36]

Although naturally AESIs, AEFIs and other coincidental events occur only after the roll out of vaccines, it is important to start surveillance even before the rolls out. This is because to detect AESIs, it is necessary to know the background rates of each AESI for comparison. Even when there is a higher rate of a possible AESI, it must be determined whether the association is causal or coincidental.

Further, when it is established that a certain AESI or AEFI has a causal relationship with the vaccine, it does not necessarily mean that vaccination must be stopped. Decision to discontinue vaccination or to exclude certain sub-populations depends on whether the AESI or AEFI results in a change in the risk-benefit profile of the vaccine.

Things to be done before vaccine roll out (and reinforcing these during vaccine roll out)

- ***Establish background rates of AESI*** – hence surveillance systems need to be established now itself.
- ***Define the list of AESIs*** [the Coalition for Epidemic Preparedness Innovations (CEPI) is developing a comprehensive list of AESIs].

- Develop harmonised case definitions of each AESI (as is being done by the Brighton Collaboration under contract with CEPI).
- As recommended by the_WHO Global Vaccine Safety Blueprint (GVSB 2.0), "Countries or regions establish either a *national expert committee for AEFIs* or regional advisory committees or equivalent objective panels with spelled out terms of reference."[39]
  - Public credibility can be optimized by ensuring that these committees are "independent of *conflicts of interest* with the ministries of health, industry and the immunization program"[39]
- "Vaccine safety *communication plans*, with clear national and subnational vaccine safety communication roles and responsibilities, should be developed to provide timely, evidence-based messaging to describe what is known, what is not known, and what is being done to fill these gaps."[36]

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Indian National Science Academy

The origin of the Indian National Science Academy (INSA) dates back to the founding of National Institute of Sciences, Kolkata in 1935. This moved to its present premises in Delhi in 1951 with its recognition by the Government of India as the premier National Science Society and in 1968, the Government of India, mandated INSA to represent in all international fora. Currently INSA is a key contributor to the scientific firmament of India and works as an autonomous institution under the aegis of the Department of Science and Technology. The objectives of INSA include, a) identifying, nurturing and promoting scientific talent; b) supporting scientific research; c) informing the government on societal aspects of science; d) develop policy recommendation through well researched, evidence based synthesis of current science; e) inform the Nation on emerging trends in science, education and research; f) develop international interfaces and g) publish scientific journals, books white paper to inform all the stakeholders.

INSA pursues its programs through its fellowship comprising close to 1000 scientists in all disciplines of Science, Engineering and Medicine. The programs are in the areas of a) international relations including representation in International Science Council and bilateral exchanges; b) identification and promotion of excellence in science; c) activities relating to interfacing science with society; d) human resource development including the conduct of DST-INSPIRE program and SERB supported future scoping and capacity augmentation program; e) initiatives on ethics, gender equality and women in science; f) History of Science and g) Scientific Publications.

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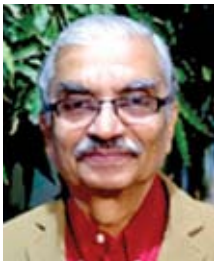

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