The Making of SARS-CoV-2 Vaccine- A Race Against Time

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Abstract

Only a few like Ophthalmologist Dr. Li Wenliang at the Central Hospital of Wuhan in Wuhan, Hubei, China could, perhaps, foresee what lay ahead for the Global Citizenry when he tried to alert Authorities about Covid 19 (C19) in late December 2019. From a National Emergency in China to an unprecedented Global Pandemic, it has etched a permanent place in the History of Global Epidemics. The WHO belatedly declared it to be a Pandemic on 11th March 2020 when the number infected had already topped 500,000. As Countries locked down their Economies in an effort to restrict the spread of C19, Scientists came together in a Competitive bid to cooperatively devise an effective therapy. The overriding concern was as to how quickly could a Vaccine be made ready given that the average timeline for Vaccine Development was 10 years. Two groups, one led by Oxford University-Astra Zeneca & the other by BioNtech-Pfizer (BTP) were first off the blocks based on what proved to be previous, path-breaking Basic Scientific Research in Molecular Biology. This Article briefly recalls those seminal contributions & highlights the beneficial offshoots of Core Scientific Research.

I. Introduction

Three years have elapsed since Covid 19 (C19) wrapped humans in its death throes in late 2019 through the 2020s taking them on a spasmodic roller coaster ride of ascending hope and descending despair before Vaccines Stopped in its tracks, lying low and weakened but determined to stay the course.

Starting with Death Zero i.e. of a 61-year-old Chinese man who succumbed to C19 on 11th January 2020, the World Health Organization's (WHO) C19 Dashboard confirms that as of 12:37 am CEST (04.07 am IST), 764,474,387 C19 cases including 6,915,286 deaths have been reported to it from across the globe. The global vaccination count then stood at 13,343,360,939.

Driven by the harsh reality of the unavailability of any prophylactic or curative and that none was expected in the near future, Countries closed inter & intra country travel. India shut down on 25th March 2020 in an attempt to isolate & contain the Coronavirus from infecting the uninfected with 'Social Distancing' becoming the byword of animated discussions and debates.

As the world grappled with the 'never before' situation, it was evident that a potent Vaccine against C19 seemed to be the only long-term viable option. However, the big question engaging Administrators, Regulators & common Citizens was when could an effective vaccine be available for the masses.

II. Background

8th May 1980 is a Red letter day in the History of Biological & Health Sciences, Medical Administration & Transnational Cooperation. The World Health Organization (WHO) declared Earth to be free of Smallpox (SP), one that killed 3 out of every 10 people it infected. SP is estimated to have killed more than 300 million people globally since 1900 and left Survivors severely and permanently scarred for life.

Chemist & Microbiologist Louis Pasteur successfully saved a young boy from certain death in 1884 after he had been bitten by a rabid dog. Pasteur vaccinated him with a live attenuated viral Rabies vaccine developed from a formaldehyde-inactivated desiccated Dog brain tissue.

In 1888, E. Roux and A. Yersin demonstrated that a toxin secreted by the Diphtheria bacillus gave rise to symptoms of Diphtheria. Thereafter, in 1890, Bacteriologists E.A.V. Behring and K. Shibasaburo's discovery of "Specific Pathogen bound Antibodies" in the Serum of vaccinated individuals led to the development of Antitoxin (Serum) based Therapy for Diphtheria. Behring was awarded the first Nobel Prize for Physiology & Medicine in 1901.

Physician Edward Jenner vaccinated 08-year-old boy James Phipps with Cowpox Virus (CP) in 1896 and then infected him with live Smallpox Virus after 02 months. Phipps'

fought off the infection formally ushering in the term 'Vaccination' derived from Vaccinia, Latin for CP. Jenner published his seminal SP Research titled *'An Inquiry into the Causes and Effects of the Variolae Vaccinae'* in 1798.

Smallpox Vaccination (SPV) debuted in Bombay (Mumbai) after Europe and the Americas. Though it reached Madras (Chennai), Poona (Pune), Hyderabad, and Surat thereafter, superstition, existing familiarity with the Variolation technique, and opposition from the existing Inoculators, i.e. the '*Tikadaars*' who feared job loss, and lack of free Vaccination facility resulted in low acceptance.

Issues such as post-vaccination- complications & deaths, ineffective immune response, and resistance to the Cow Pox-based vaccine from a section of the Cow venerating Hindu community confronted Vaccination campaigns around 1850. Ineffectual implementation rendered 'The Compulsory Vaccination Act, 1892' infructuous.

Ukrainian Zoologist, Dr. Haffkine demonstrated the efficacy of the Cholera vaccine developed by him post-trials in Agra, Uttar Pradesh in 1893. The proliferation of Plague disease led to the enactment of the Epidemic Act of 1896. His Laboratory, (now named Haffkine Institute) at the Grant Medical College, Mumbai developed Plague Vaccine, the first vaccine made in India in 1897. Crucially, Haffkine's work showed that Exalted or Strengthened culture of Polyclonal antibodies resulted from the repeated passage of pathogenic Bacilli through an Animal's body. This culture could then be Thermally Attenuated for Human Safety.

III. The Immune System

Viruses, Bacteria, Pathogenic Fungi, and Parasites are the 04 broad & major categories of Pathogens or disease-causing Microorganisms.

The term 'Immunology' was coined by Russian Biologist I.I. Mechnikov who conducted advanced Research on Immunology. Metchnikov, Behring & Ehrlich's discoveries of Phagocytosis (cells engulfing & destroying intruding Pathogens) and Microbial Toxin Neutralizing Antibodies respectively laid the groundwork for the Field of Immunology. Mechnikov was recognized with the Nobel Prize for Physiology or Medicine in 1908.

Subsequently, discoveries of Bacteriolysis by Complement[#] and Opsonization by Antibodies provided the first evidence of networked functioning of the Acquired and Innate Immunities. Explanation about Pathogenic consequences of Immune Response, and Hypersensitivity types followed. Delineation of Antibody Structure by Chemists Rodney Porter and Gerald Edelman won them the Nobel Prize in 1972.

The 78 major Organs in the Human Body are associated with one or more specialized functions making the Body operate as a composite Biological Machine. Groups of Organ Systems each composed of Organs & Tissues function together as a Unit to produce and sustain life.

One of these, the Immune System (IS), is a complex, widely distributed, and rigidly regulated, overlapping Antibody Effector Functions, a critical component of the Humoral Immune Response, and an essential link between Innate and Adaptive Immunity.

Fig 1 Components of Human Immune System – Page 542

The majority of the Functions are activated against substances it deems foreign to the body like Allergens, Bacteria, Viruses, etc. Activation occurs over the Constant (Fc) Region of the Antibody on account of its ability to network with Complement Proteins and Specialized Fc-receptors.

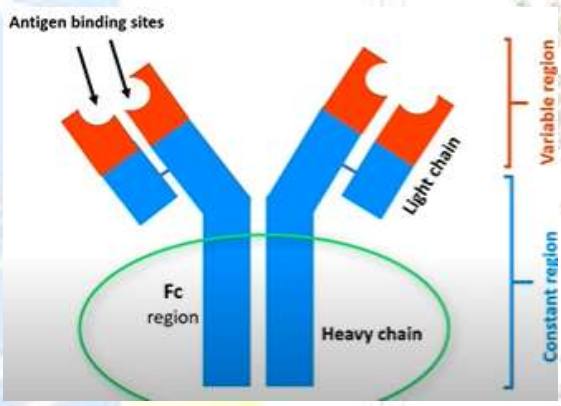


Fig 2 Antigen Binding Site in the Constant (Fc) Region. Image courtesy Pharmacology Animation Book

Innate Immunity (II) & Adaptive Immunity (AI) are the synergetic tools that the IS employs to defend against Structural & Chemical Barrier breaching pathogens. The II, the first immunological mechanism is an Antigen independent (nonspecific) non-Immunologic Memory, Rapid Immune Response that is initiated minutes or hours after Intrusion. Antigen-dependent & Antigen-specific AI is abled by Immunologic Memory enabling the host to mount a more rapid, robust, and efficient immune response in cases of subsequent Antigen Exposures. Defects in either system can provoke illness or disease like Inflammation (Rheumatoid Arthritis), Autoimmune Diseases (Psoriasis), Immunodeficiency Disorders like Chronic Granulomatous Diseases or CGD that make afflicted humans highly susceptible to Bacterial & Fungal Infections, and Hypersensitive to reactions like Food Allergy.

Four types of defensive barriers i.e. Anatomic (Skin & Mucous Membrane), Physiologic (Temperature, low pH & Chemical Mediators), Endocytic & Phagocytic, and Inflammatory make up the II. Pattern Recognition Receptors (PRRs) allow Immune Cells like Dendritic cells, Macrophages, Monocytes, Neutrophils & Epithelial cells to express Proteins that detect molecules that are typical of a Pathogen**.**

Fig 3 Defensive Barriers- Page 543

The two molecule classes are Pathogen-associated Molecular Patterns (PAMPs) linked to Pathogenic Microbes & Damage-associated Molecular Patterns (DAMPs) connected with the Host cell parts that get released on cell damage or on death.

The IS records the makeup of every Microbe it has confronted & destroyed in White Blood Cell (WBC) Types (B-lymphocytes & T-lymphocytes) known as Memory cells arming it to recognize and annihilate the microbe should it reenter in the future.

Small Proteins, Cytokines are produced mainly by Activated Macrophages, Monocytes, and T cells. Cytokines regulate the immune responses like mobilizing Multiple Defense Systems, and Switching on Native Cellular Responses against Infections within the body, control Inflammatory and Hematopoietic processes, and may induce fever.

Primary & first-line Cytokines against Bacterial infections include Tumour Necrosis Factor (TNF), Interleukins 1 (IL-1) & 6 (IL-6), while those for Viruses Are Interferons (IFNs)- α, β, and γ, T cells & NK cells, TNF- α, IL-1, and IL-6. Local inflammation is a natural way of getting rid of Pathogens. Dysregulated Production of such Inflammatory Cytokines is often associated with Inflammatory or Autoimmune disease, making them important therapeutic targets.

The Complement System (CS) is made up of a large number of distinct Plasma Proteins that react with one another to Opsonize or Coat Pathogens & Induce a series of Inflammatory Responses that help to fight infection. This Coating helps Immune cells to Phagocyte Bacteria i.e. Target, Encompass, and Kill the Microbes.

The Phagocytic action of the II Response involves Phagocytes (02 main cell types, short-lived Neutrophils & long-lived Macrophages), Dendritic cells, Mast cells, Basophils, Eosinophils, Natural Killer (NK) cells, and innate Lymphoid cells. These Phagocytose (engulf) microbes and kill them through multiple Bactericidal pathways.

In addition, Neutrophils contain Granular & Enzymatic pathways that assist in the elimination of pathogenic microbes by clearing dead cells or Antibody complexes and removing foreign substances present in Organs, Tissues, downloading PDF (*viscous fluid composed of Plasma suspended cellular elements*) Blood, and Lymph. Macrophages, unlike Neutrophils, are also involved in Antigen Presentation to T cells. The II can also activate the AI response through the mobilization and activation of Antigen-presenting cells (APCs).

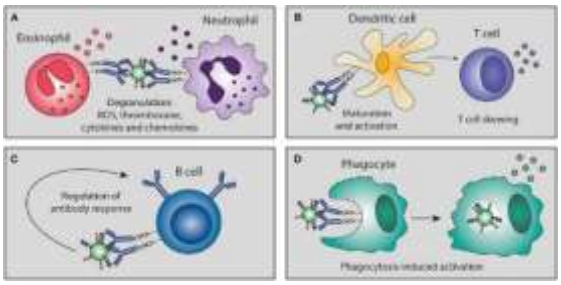


Fig 4 Phagocytosis Image Courtesy Frontiers in Immunology

IV. Timelines & Success Rates in Vaccine R & D

History recorded Timelines in Vaccine Developments are 4 years for Mumps, 13 years for Polio, 17 years for HPV, 27 years for Flu, and 28 years for Chickenpox. Struck's Analysis shows that full-scale Vaccine Development has 10-year Timelines on average. In this only half of the Preclinical Vaccine Candidates make it through the entire process. Transition probabilities for progress from Phase I to Phase II and from Phase III to Registration are 72% & 71% respectively.

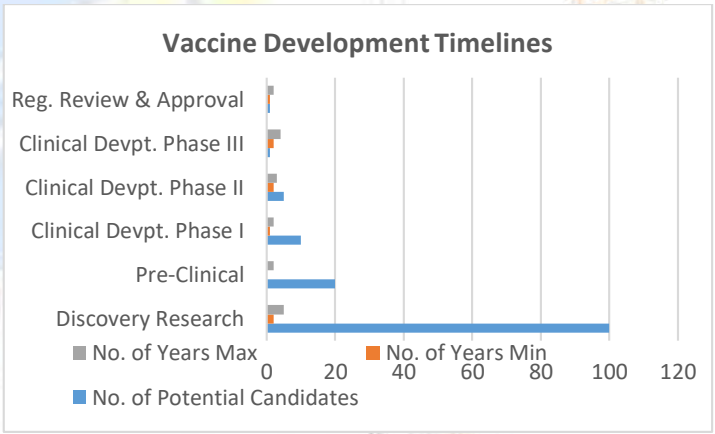


Fig 5 Vaccine Development Timelines

Hyper-attenuation of Microbes, Insufficient Immunogenicity, or Insufficient Attenuation revealed during the first Human Exposure in Phase I is responsibmle for the failure of Vaccine candidates.

Struck opines that the preclinical Phase herein is very demanding and time-consuming - establishing Cell Banks, Adjuvant studies, Evaluating Inactivation and Detoxification, and System of Lot Release as per Internationally Accepted Standards. These apart, the preparation of Reference materials is an onerous and time-consuming affair.

The lower transitioning probabilities to Phase III are due to the presence of multiple variables in Phase II Trials consisting of Vaccine Administration to a larger number of people, different Dosing Schemes & Dosages, multiple routes of administration, and different Adjuvants.

Surrogate Efficacy markers like Serum Titers, Animal Challenge Data, or Neutralizing Antibodies may be misleading as these are outcomes of trials on Healthy Volunteers and explains the higher probability of failure of Candidates to progress from Phase III to Registration as the recipients are from the general population.

For Live Attenuated Vaccines, Genetic and Environmental Stability are established, and Environmental Detection Methods have to be refined prior to their usage.

Fig 6 Stages in Vaccine Development- Page 544

V. Payoffs & Spinoffs of Basic Scientific Research

Developing an Effective Vaccine within 12 months of the Novel Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2) Genome being made public is a remarkable Scientific tour de force. What has not been widely publicized, however, are the existing Publicly Funded Research Innovations in Fundamental Immunology that proved critical in making this timeline a reality.

K. Karikó and D. Weissman's seminal paper in 2005 showed that modification of select nucleosides could suppress the body's recognition of synthetic RNA, avoiding an overwhelming immune response. Stem Cell Biologist D. Rossi & his colleagues at Harvard and MIT co-founded Moderna in 2010 hoping to use this knowledge for growing Stem Cells. Ugur Sahin & Özlem Türeci, the Turkish-German Physicians and co-founders of BioNTech, hired Karikó to oversee the development of mRNA technology in preparation for the future.

Working with an unsuccessful version of a 1966 vaccine against the Respiratory Syncytial Virus (RSV), B. Graham & Associates isolated the RSV Fusion protein, described its structure, and found the pre-fusion antibodies to be considerably more effective than the post-fusion antibodies in provoking an Immune Response. Graham and colleagues published a detailed description of the complete pre-fusion Spike Protein of the SARS and Middle East Respiratory Syndrome (MERS) related virus, the Human Betacoronavirus HKUI1 in 2016 riding on USA Government's Support channeled via the National Institute of Allergy and Infectious Diseases (NIAID).

In 2017, Researchers at CureVac, a Biotech company demonstrated that an mRNA vaccine could induce functional antibodies against a Viral antigen of the Rabies Virus. Similarly, Moderna working on Viral Diseases Zika and Chikungunya validated that mRNA sequences could be used to coax the secretion of a Human protein and potentially Scale Antibody response against a specific target in the human body. Both were funded by the US Government. These foundational advances had, therefore, laid the base for the race for a C19 vaccine.

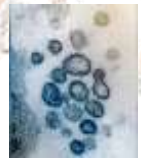


Fig 7 SarsCov2 Virus under Electron Microscope. Image courtesy CNET/NIAD

Majority of the C19 vaccines currently in use target the Spike protein of the SARS-CoV-2 virus—a Prefusion Protein the Virus uses to infect host cells.

Isolating & Preparing the Spike Protein for a Vaccine is a tedious process with Researchers having to modify it & then multiply it. Researchers who developed these mRNA Vaccines started with the Genetic Structure of the Virus. Instead of Assembling & Purifying that Protein in the Lab, they identified part of the Protein that creates it. By synthesizing mRNA and using that as a Vaccine, they saved large amounts of Time & Money. Once inside the body, the IS recognizes the threat & acts on it. It is obvious that mRNA vaccine development would have been extremely difficult within a year had these Advances not been available.

VI. The Race to Produce a Vaccine Against C19

A Virus is an infectious Microbe consisting of a segment of Nucleic Acid (either DNA or RNA) surrounded by a Protein coat with some encapsulated in nanometre-sized Capsids. Viruses cannot self-replicate. Viruses need to infect Host cells and use their machinery to make copies of itself. Capsids have complex mechanical properties and encapsidate the viral genome in one host, transport it, and subsequently release it inside another host cell. Viruses like the Coronavirus with RNA are termed RNA viruses.

On the basis of Genome type, single-stranded RNA viruses are classified into +ve & –ve sense RNA viruses. The +ve sense RNA virus is also referred to as Sense strand or +strand, while on the other hand, -ve sense RNA is also referred to as Antisense or -strand.

Scientists use Genome Sequencing techniques in Molecular Biology to view an individual's makeup and the Proteins they encode to identify Genetic changes, Non-coding DNA, their association with diseases and Phenotypes, and identify potential targets for Drugs. Rapid advances in next-generation sequencing technologies like Laboratory Tools, Computational Methods, and Strategic Approaches have helped speed up Sequencing.

Virologist & Evolutionary Biologist Edward Holmes, a member of the Shanghai Public Health Clinical Center & School of Public Health Consortium posted the sequence of the C19 Genome on an open-access site, virological.org. The consortium also deposited the sequence in GenBank, part of the International Nucleotide Sequence Database Collaboration (INSDC) that stores an annotated collection of all publicly available DNA sequences.

Analysis commenced immediately. Evolutionary biologist Andrew Rambaut of the University of Edinburgh reported that the virus is 89% similar to a Severe Acute Respiratory Syndrome (SARS) related member of the subgenus *Sarbecovirus*es under *Betacoronavirus* genus. It was also found to be most distant from the four known SARS-related Bat Viruses capable of infecting humans.

Fig 8 Different Vaccine Strategies – Pages 545, 546

Multiple Scientific Teams from Industry & Academics explored different Approaches in their choice of a Platform for C19, a critical aspect of Vaccine Development. Essentially, the principal approaches i.e. the Viral Vector and the Messenger RNA (mRNA) Platforms involved remodeling existing Platforms. The BTP mRNA platform took advantage of years of research experience on the mRNA platform for a potential HIV vaccine & other conditions associated with Diabetes.

Fig 9 Different Types of Vaccine Platforms- Page 546

The Vaccine devised by J & J is based on a Viral Vector Platform that was earlier used for Vaccines like Ebola and utilized adenovirus 26 (Ad26). Past experience in engineering Ad26 helped them initiate the C19 program quickly. The AstraZeneca Vaccine approved in India & other Nations is also based on a Viral Platform that consists of a Replication-Deficient Chimpanzee Adenoviral Vector ChAdOx1, containing the SARS-CoV-2 Structural Surface Glycoprotein Antigen (spike protein; nCoV-19) Gene.

For C19, Researchers isolated part of the Coronavirus's Genetic Sequence that Codes for the Spike protein. They then inserted the code-containing molecule into an extremely tiny Drug Delivery System, the Lipid nanoparticle that can invade human cells. Once inside, the Ribosomes use the code to create a Protein chain resembling the Coronavirus Spike Protein. Display of the lookalike Protein prompts the IS cells to respond as if it were Antigen in a traditional vaccine. Helper T-cells signal B cells to develop Antibodies and help develop Cytotoxic T-cells that directly destroy infected cells.

mRNA Vaccines work by instructing Cells to produce copies of specific Proteins in the Pathogen. Adenovirus Vectors elicit a strong immune response in Humans. Scientists used an adenovirus strain found only in Chimpanzees, as pre-exposure (*higher probability for Human Ad*enoviruses) would have rendered the vaccine unhelpful as the body would have developed immunity to it.

Details of some C19 Vaccines developed (prior expertise, Platform, Protein Targeted, Mechanism of Action, Efficacy, Pros & Cons, etc. are elucidated in Fig 8 below

The BTP COVID-19 Vaccine received the first Emergency Use Authorization from the FDA & the European Medicines Agency (EMA) on 11th Dec. 2020 & 31st Dec. 2020 respectively for individuals ≥16 years of age based on Safety & Effectiveness data from a Randomized, Controlled, Blinded Ongoing Clinical trial of thousands of Individuals.

In India, the National Institute of Virology, Pune first isolated the COVID-19 strain from asymptomatic patients. In May 2020, the strain was sent to BBIL, where the researchers studied the strain to develop an inactivated vaccine, now known as COVAXIN.

WHO provided EUL for 02 versions of the AstraZeneca/Oxford COVID-19 for a global rollout through COVAX. Serum Institute of India was one of the only two global manufacturers. The Indian Drug Regulator, CDSCO approved this Vaccine for use in India on 31st December 2020 with the first dose being administered on 16th January 2021. 2.2 Billion doses including the 1st, 2nd & the Precautionary (booster) of the currently approved vaccines have been administered as of 4th March 2023.

VII. Conclusion

The Spiraling Death Count across Nations during the 1st phase of C 19 Infections was met with Several Stringent Administrative Measures, some common & others Country or Region Specific. The sequential banning of international flights and the race to get their own citizens out from severely affected countries was accompanied by the imposition of lockdowns resulting in unprecedented levels of Socio-Economic Chaos. As Hand washing, Facemasks, and Physical Distancing became

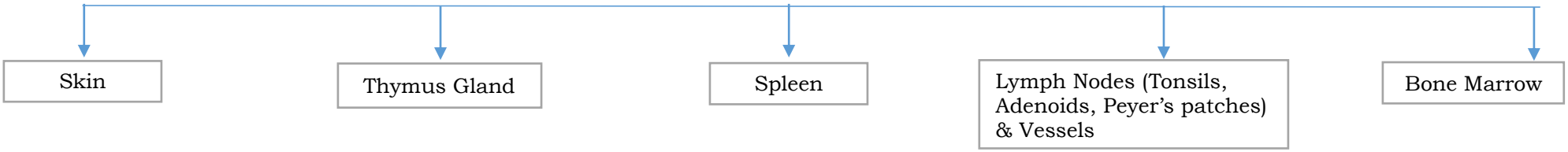
the new norm, the panicked citizenry rushed to act on unproven advisories on myriad Drugs & Curatives. Not to be outdone, the Media made a beeline for multi-hued "Experts" hoping to feature them in their "Scoops" that often involved finger-pointing at National & International Health Administrators.

All this while, a sane group of Scientists, Technologists, and Regulators wracked their Brains on ways to get people out of the quagmire the modern Human Civilization had brought upon itself with their greed to dominate. The realization that Pandemics are borderless made global citizens realize the need of the hour was to cooperate for the development of one or more potent therapies.

This write-up has tried to retrace the steps in this journey that reinforce the intrinsic values of Basic & Applied Techno-Scientific Research. C19 continues to haunt the human populace even as life has returned to near normal worldwide.

It is hoped that the cumulative experiences drawn thereupon would have made us all realize that misadventures with Nature are bound to have grave consequences and a repetition of which may put us on the path of "*No Return"* because Modern Science understands only a small fraction of the Scientific Mysteries the Universe may be harbouring in its bowels.

HUMAN IMMUNE SYSTEM



	Nature of Defence	Type	Component	Methodology of Action
Innate System (IIS)	1st Line	Antigen Independent Non-specific Non-Immunologic Rapid Response- Immediate	Surface (Anatomic – Skin & Mucous Membrane), Physiologic (Temperature, Low pH, Chemical mediators), Inflammatory & Complement System (CS), Endocytic & Phagocytic	1. Thick & sticky Mucous coats body openings like the mouth. 2. Microfiber Cilia in the lungs, & Epithelial cells lining hair in the Nasal cavity trap aerial pathogens. 3. The Spleen filters circulating blood to detect pathogens. Activates IS Cells to multiply & neutralize pathogens 4. Commensal Bacteria train Immune cells to function properly and calibrate the activation threshold of innate antiviral immunity 5. Non-specific Neutrophils access invader location, surrounds & ingest them (phagocytosis). 6. Effector Lymphocytes, the Natural Killer (NK) cells. Interact reciprocally with Dendritic cells (DCs), Macrophages, T cells, and Endothelial cells. NKs attach to Microbes and release chemicals to kill them allowing the AIS to mount a targeted response.
Adaptive System (AIS)	2nd Line	Antigen Dependent Antigen-Specific Immunologic Memory linked Rapid, Robust, Effective Response- within Minutes/ Hours	Lymphocytes (*a White Blood Cell –WBC),* B cells & T cells generate from Bone Marrow Stem cells & mature in Marrow & Thymus respectively.	1. Dendritic cells, Macrophages, and B cells also serve as Antigen Presenting cells (APCs). 2. DCs monitor pathogens in tissues, phagocytose, break them & place these on the surface as a signal (Antigen) while at the nearest lymph node to activate T cells. 3. MHC II molecule embedded Helper T cells, a lymphocyte responds to APCs. 4. Cytokines are small proteins that bind to a Cell surface having a matching marker termed Receptor. A single Cytokine can attach to more than one Receptor. 5. Primary & 1st line Cytokines for Bacterial infections include Tumour Necrosis Factor (TNF), Interleukin 1 (IL-1) & 6 (IL-6), those for Viruses is Interferons (IFNs)- α, β, and γ, T cells & NK cells, TNF- α, IL-1, and IL-6. 6. Lymphatic Fluid transfers pathogens & debris in tissue to nodes for filtration.

Fig 1: Components of the Immune System

Barrier		**Mechanism**
Type	**Detail**	
Anatomic	Skin	Acts as a Mechanical Barrier to retard Pathogen Entry
		Acidic Environment (pH 3-5) retards Microbial Growth
	Mucous Membrane	Normal Flora Competes with Microbes for Sites of Attachment
		Cilia Propels Microbes out of the Body
		Mucous Traps foreign Microbes
Physio-logic	Temperature	A rise in Body Temperature inhibits the growth of some Pathogens
	Low pH	Acidic pH in the Stomach kills undigested Microbes
	Chemical Mediators	Lysozome Cleaves Bacterial Cell wall
		Interferon Induces Antiviral Defenses in uninfected Cells
		Complement Lyses Microbes or facilitates Phagocytosis
Phagocytic /Endocytic		Different Cells internalize (Endocytosis) & break down Foreign macromolecules
		Specialized cells (Blood Monocytes, Neutrophils, Tissue Macrophages) internalize (Phagocytose), kill & Digest whole organisms
Inflammatory		Tissue Damage & Infection induces leakage of Antibacterial Serum Protein containing Vascular Fluid. This prompts Phagocytic Cell Influx into the affected area

Fig 3 Defensive Barriers Courtesy Marshall et all, An Introduction to Immunology & Immunopathology

Stage		Candi-date (Nos.)	Min	Max	Activity	Objective / Rationale
Discovery Research		100	2	5	Spores, Attenuated Viruses/ Bacteria, Bacterial Toxins, Pathogen Originating Substances are Evaluated	Antigenic Potential
Pre-Clinical		20	0	2	Experimentation on Cells, Tissues, Rodents & Animals	Check Safety & Efficacy, Route of Administration, Effective Dosage & Efficacy in Provoking an Immune Response (Immunogenicity).
Clinical	Phase I	10	1	2	Vaccine is tested on 20-80 Healthy Volunteers.	Reveals Efficacy, Side Effects, Dosage Sufficiency & Immune Response (Antibody Production).
	Phase II	5	2	3	Hundreds of Volunteers are Randomly Chosen to receive the Vaccine or a Placebo (substances without any therapeutic effect, used as a Control)	Track Vaccine Efficacy, Dosage, Duration of Immunization, and Delivery Mode- Oral /Subcutaneous, Intramuscular/Intradermal/Intranasal.
	Phase III	1	2	4	Thousands of Volunteers Randomly Assigned to Experimental/ Placebo Groups.	Test Efficacy & Safety in a larger Group, particularly for the Intended Population. Helps detect rare side effect (s)
Regulatory Review & Approval		1	1	2	Vaccine is Approved by Regulators	Vaccine proven to be Safe & Reliable & Benefits outweigh the Risks
Pharmacovigilance					Manufacturer Monitors Efficacy to Avoid Adverse Events.	Regulators Monitor the Entire Production Process.

Fig 6: Stages in Vaccine Development

	Vaccine Class	Used against	Type	Mechanism of Action	Immune Response Generated	Tenure of Immunity	Constraints/ Benefits	Examples
WHOLE MICROBE	Live Attenuated	Mainly V	Weakened Virus Infects Cells, replicates	Induces B-Cell Memory	Strong	Lifelong vide 01/02 Doses	Causes Nil/ mild Disease. Refrigeration needed. Unsuitable for Weakened IS/ long-term health condition.	MMR, Rotavirus, Smallpox, Chicken Pox, Yellow Fever
	Inactivated	B, V	Dead Virus		Less Strong as compared to LA	Needs follow-up Doses over time.	Fewer Side Effects	Hepatitis A, Influenza, Polio, Rabies
PARTS OF MICROBE	Subunit	B, V	Isolated Surface Antigen Triggers I.R.		Very Strong	Needs follow-up Doses over time.	Suitable for those with weakened IS/long-term health conditions. Fewer Side Effects	Hib, Hepatitis- B, HPV, Pertussis, Pneumonia, Shingles, Meningitis
	Recombinant	B, V	Protein Expressing Gene placed inside a Cell	IS recognizes the Protein & Generates an I.R.				HPV
	Conjugate	B, V	Outer coat Antigen part Conjugated (linked) to Strong Carrier Protein		Generates aggressive I.R			Hib
	Polysaccharide	B, V	Outer layer Polysaccharide Conjugated (linked) to Strong Carrier Protein		Generates aggressive I.R.			Pneumococcal Conjugate Vaccine
TOXIN BASED	Toxoid	B, V	Toxin from disease-causing parts of B/V.		Strong Toxin-targeted I.R.	Needs follow-up Doses over time.		Tetanus, Diphtheria
mRNA BASED	mRNA	V	mRNA piece Analogous to Viral Protein	Triggers Cellular & Humoral Immunity Response	Robust		Require Refrigeration. Shorter Manufacturing Time & Cost. Safe, is Quickly Designed, Tested & Mass Produced.	Gemcovac-19
MOD. V	Viral Vector	V	Modified V acts as Vector		Strong	Lasting		Ebola, C19
ENCODED DNA PLASMI	DNA	B, V	Injects DNA to create Antigens	IS recognizes DNA & mounts I.R.	More effective than Protein / Antigen based ones	Long-term		C19 (ZyCov-D)

RECOMBINANT PROTEIN	Recombinant Vector	V	Engineered V carries extra genes from Pathogen	Extra Genes produce Proteins that I.S. recognizes & protects against	Strong	Long-term		Hepatitis B

Legend: B→ Bacteria; V→ Virus

Fig 8 Different Vaccine Strategies

Entity	Expertise gained from working on	Platform	Action	Targeted Protein	Viral Vector	Efficacy % in Phase III trial	Pros/ Cons
BioNtech -Pfizer (BTP)	Vaccines for HIV & Diabetes associated conditions	mRNA	Instructs Cells to produce copies of Protein & prompting Antibody creation			95 after 28 days of 1st Dose.	Required to be stored at 70°C±10°C.
Johnson & Johnson (JJ)	Vaccines for Ebola & others	Viral Vector	Elicits IS response by presenting copies of Viral Antigen	Spike Protein-nCoV-19*	Adenovirus 26 (Ad26).		
Moderna (M)	Vaccines for MERS, SARS, Influenza, HIV & Hep. C	mRNA	Instructs Cells to produce copies of Protein & prompting Antibody creation			94.1	
Astra Zeneca (AZ)	Vaccines for Zika, Cancer & HIV	Viral Vector	Elicits IS response by presenting copies of Viral Antigen		Chimp Adenovirus ChAdOx1	70 after 22 days of 1st Dose.	

*→ **SARS-CoV-2 Structural Surface Glycoprotein Gene**

Fig 9 Different Types of Vaccine Platforms

VIII. References

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