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Intranasal COVID-19 Vaccine - Targeting Nasal Mucosal Microenvironment

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Abstract

COVID-19 is a highly pathogenic and transmissible viral disease that has threatened the human health and safety worldwide. COVID-19 infection is caused by Severe Acute Respiratory Syndrome Corona Virus -2 **(**SARS-CoV-2**)** and affects mucosal surfaces. Studies have stated high viral load in nasal cavity as compared to throat. Thus, nasal cavity serves main target for viral entry, shedding and transmission.

Therefore, a vaccine administered through nasal route could be highly effective in controlling disease manifestation and progression. Animal studies conducted on the efficacy of the intranasal vaccine state that, the change in the route of vaccine administration initiates mucosal immune response and reduces the nasal airway mediated viral shedding. These intranasal vaccines are administered through a nasal spray and thus can be self-administered. Therefore, an intranasal vaccine is easy to administer among larger population and effectively reduces rate of COVID-19 transmission.

Key words – COVID-19, Intranasal Vaccination, Nasal microenvironment, SARS-CoV-2.

Introduction

Novel corona virus is a positive sense RNA virus having a wide range of natural hosts and affects multiple systems1. This dreadly virus has led to the emergence of COVID-19 pandemic which has posed great threat to the entire human race. The virus has been named Severe Acute Respiratory Syndrome Coronavirus 2 **(**SARS-CoV-2**)** by the International Committee on Taxonomy of Viruses **(**ICTV**)**, which determined that the virus belongs to the Severe Acute Respiratory Syndrome-related coronavirus category **(**2**)**.

Structure

SARS-CoV-2 is a member of the order Nidovirales, family Coronaviridae, subfamily Orthocoronavirinae, which is subdivided into four genera, viz., Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus The genera Alphacoronavirus and Betacoronavirus originate from bats, while Gammacoronavirus and Deltacoronavirus have evolved from bird and swine gene pools. The new SARS CoV-2 belongs to betacoronavirus1. The corona virus genome is comprised of 30,000 nucleotides. It comprises of four structural proteins and several non-structural proteins **(**nsp**)** **(**Figure -1**)**.

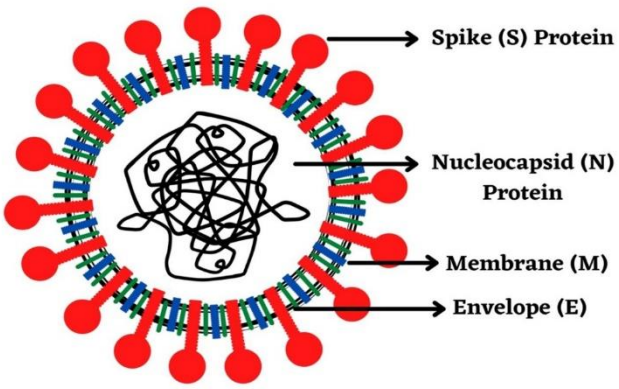


Figure 1 – Structural Proteins of SARS-CoV-2; Nucleocapsid **(**N**)** protein; Membrane **(**M**)** protein; Spike **(**S**)** protein.

Mucosal Immune System and COVID19

Corona virus infection can be transmitted by both symptomatic and asymptomatic individuals before onset of symptoms. Symptomatic patients may transmit disease via large droplets generated during coughing and sneezing3. It can also be transmitted by touching surfaces contaminated by virus. Studies have shown high viral load in nasal cavity compared to that of throat in COVID-19 positive individuals. Studies have shown no difference in viral load between symptomatic and asymptomatic or minimally symptomatic patients showing same potential for disease transmission **(**4**)**.

The Spike protein aids in entry of virus into the host and is capable of inducing host immune response. The crown like appearance to the virus is provided by the structure of S protein. The SARS-CoV-2 gains entry via mucosal route like nasal mucosa, oral cavity or genital tract5. Therefore using a mucosal route of vaccination is of great importance and provides a rational reason to induce a protective immune response.

The mucosal immune activities of the body are associated with lymphoid tissues i.e. mucosa-associated lymphoid tissue **(**MALT**)**, which is present in mucosal tissue in the nose, lungs, gastrointestinal tract and vaginal/rectal surfaces. MALT is divided into sub-compartments based on location6 **(**Figure-2**)**.

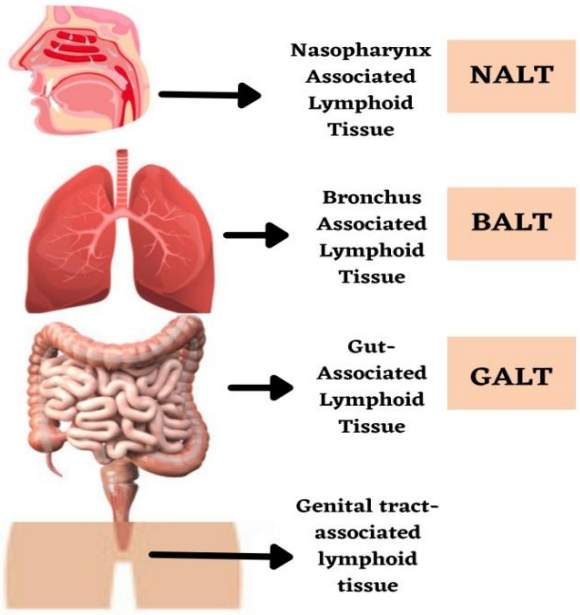


Figure 2 – Divisions of Mucosa Associated Lymphoid Tissue - MALT is divided into subcompartments based on location as NALT – nasopharynx associated lymphoid tissue.; BALT- bronchus associated lymphoid tissue.; GALT - gut-associated lymphoid tissue; Genital tract-associated lymphoid tissue.

Routes of mucosal vaccination within the mucosa-associated lymphoid tissue **(**MALT**)** are intranasal, sublingual, oral, rectal and intravaginal. Mucosal immunization has been utilized successfully in human vaccination. The human mucosal immune system is large and protected by immune cells that populate the region along the mucosal surfaces. The immune cells along with epithelial cells and mucus acts as physical barrier to the pathogen before it gains access to the underlying tissues and induces harmful effects7. Since mucosal surfaces are intial point of contact to corona virus, using a mucosal route of vaccination could be beneficial in halting the disease onset as well as progression. Nasal delivery of vaccine offers an easily accessible route to the immune system8. The mucosal vaccination delivery routes such as oral, nasal, pulmonary, conjunctival, rectal and vaginal mucosa have been regarded as potential vaccine delivery sites. However for practical and cultural reasons, researchers have tended to focus only on oral, nasal, and pulmonary administration **(**9**)**.

Single-cell RNA sequencing revealed that the nasal ciliated epithelial cells and goblet cells express highest levels of angiotensin-converting enzyme 2 **(**ACE2**)** and the cellular serine protease TMPRSS2 receptors. Both of these are main entry receptors for SARS-CoV-2 via interaction with viral spike protein13,10. This implies that nasal mucosa acts as a primary interactive medium for SARS-CoV-2 acting as loci of infection and disease transmission. It also aids in the viral shedding**(**10**)**.

Co-expression of entry receptors in other barrier surface tissues like esophagus, ileum and colon could support the findings of viral fecal shedding observed clinically11. This could explain the reason for potential fecal–oral transmission. The co-expression of receptors in superficial conjunctival cells could explain an ocular phenotype observed in a small portion of COVID-19 patients with the potential of spread through the nasolacrimal duct12. Further investigations are required into alternative transmission routes. As SARS-CoV-2 is an enveloped virus, its release from the host cell does not require lysis of the infected cell. The virus gains entry through the nasal mucosa, targets the goblet cells and utilizes its secretory pathways and sustain in these goblet cells during presymptomatic stage. These findings imply that the nasal cavity is not merely the site of entry of virus but also plays central role in viral shedding and transmission, serving as main target of SARS-CoV-210. Any drug/vaccine administered intranasally could prove to be highly efficacious in halting disease manifestation, transmission and progression. In order to propagate in this direction, it is imperative to understand the initial Host-Viral interaction in Nasal and NALT microenvironment, along with its outcomes **(**Figure 3**)**.

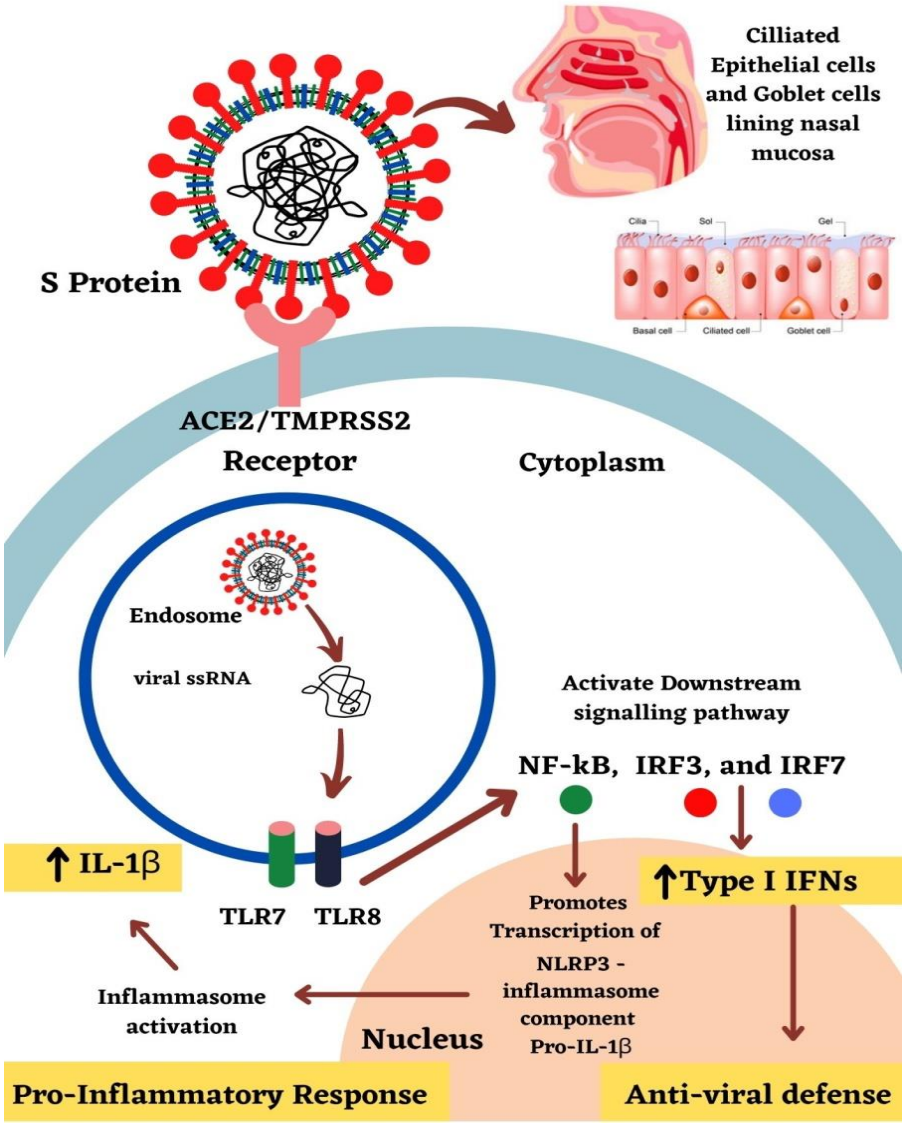


Figure 3 - Host – Viral interaction in nasal mucosal microenvironment- The SARS-CoV-2 interacts by binding to ACE2 **(**Angiotensin Converting Enzyme 2**)** and TMPRSS2 seriene protease receptors present on nasal epithelial and goblet cells via viral S **(**spike**)** protein13,10. This Viral S protein- Host - Receptor interaction makes way for entry of virus into the host cell where it gets bound into the endosome. Further, the viral ssRNA **(**Single Stranded RNA**)** is released which is recognized by TLR7 and TLR8 **(**Toll Like Receptors**)** leading to activation of several downstream signaling pathways like NF-kB, IRF3 and IRF7. Transduction pathway mediated by NF-Kb promotes transcription of NLRP3, a component of Inflammasome along with proinflammatory cytokine like pro-IL-1β. This Inflammasome activation promotes maturation of pro-IL-1β to IL-1β 13,15. This is the pathway for increased pro-inflammatory response. On the other hand NF-Kb Coordinates with IRF3 and IRF7 to increase the expression of Type I IFN. This Type I IFN acts in autocrine manner and leads to activation of IFN stimulating genes, which

in turn increases production of interferons. This has a significant role to play as it provides Anti-Viral Defense **(**13,17,19, 20**)**.

The pro-inflammatory response and anti-viral defense are significant outcomes of interactions between SARS-CoV-2 and Nasal mucosal cells. The nasal and NALT microenvironment shows a highly inflammatory form of programmed cell death known as pyroptosis which is usually seen in case of cytopathic virus infection. SARS-CoV-2 being one among them, the inflammasome activation during host-viral interaction leads to pyroptosis of the host cell which generates high amount of proinflammatory cytokines like IL-1β14,15. The PRRs like NLRs, TLRs play significant role as in they recognize DAMPs, PAMPs, Viral RNA , DNA and triggers the immune response to weaken the virus and its effects13,14,15,16. A group of Researchers used reconstituted human airway epithelia to isolate and then characterize the viral infection kinetics, tissue-level remodeling of the cellular ultrastructure, and transcriptional early immune signatures induced by SARS-CoV-2 in a physiologically relevant model. The results stated that the transcription of proteins belonging to NF-kB and TNF alpha pathway were significantly higher in nasal epithelium compared to that of bronchial epithelium. This confirms that these intricate differences in immune responses in upper and lower airways existed both in terms of kinetics and intensity and lead to putative intrinsic differences in early response to SARS-CoV-216.

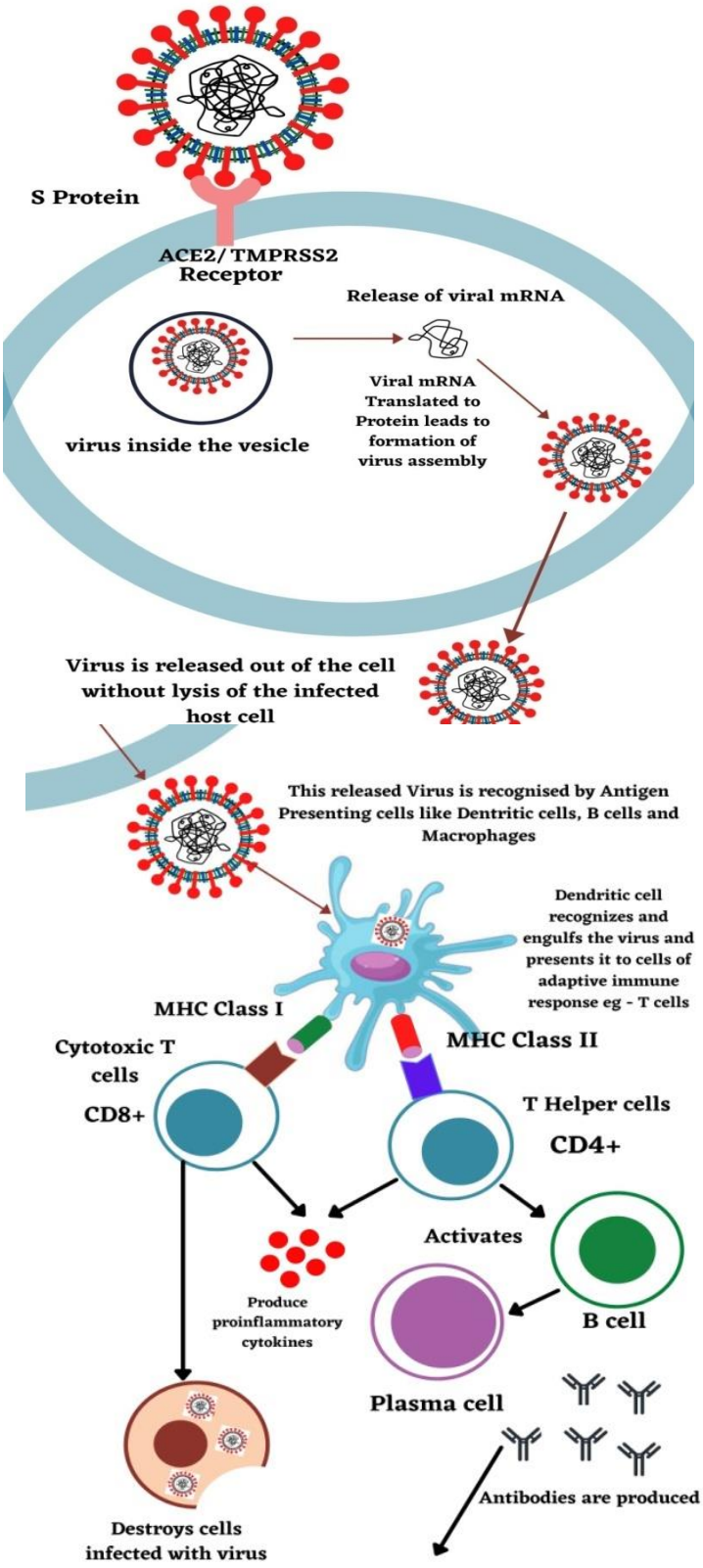
The severity of immune response decides fate of the disease, for example- moderate immune response provides protection against pathogen, on the other hand hyperactive immune response leads to over production of mediators causing destruction of host tissues, making way towards disease progression. One such hyperactive immune response leads to “Cytokine Storm”. It may also be due to impaired immune response14,17. It can be hypothesized that the nasal mucosa controls disease manifestation, transmission and progression. Therefore, nasal route of therapeutic administration could be promising.

Intranasal Vaccination for COVID19

All vaccines aim at providing protection from invading pathogen by introducing either a part of an antigen, its inactive form or nucleic acid into the host. This activates the immune response and antibodies are produced against the virus, which will prevent person from getting infected and if infected helps him combat the disease.

The first line of defense against any pathogen including virus is constitutes innate immune response. The cells of innate immunity include myeloid cells **(**monocytes, macrophages, dendritic cells **(**DCs**)**, and granulocytes**)** and innate lymphoid cells **(**ILCs**)**18,19,21. These cells orchestrate the direct clearance and eradication of pathogens, and contribute to the generation of long lived adaptive immune responses22,23,24. The type of activated immune cell, the nature of the activation signal, and the

identity of the activated immune cell receptor**(s)** govern the intensity and quality of the immune response to invading pathogens. This forms the basis of vaccine development **(**Figure - 4**)**.



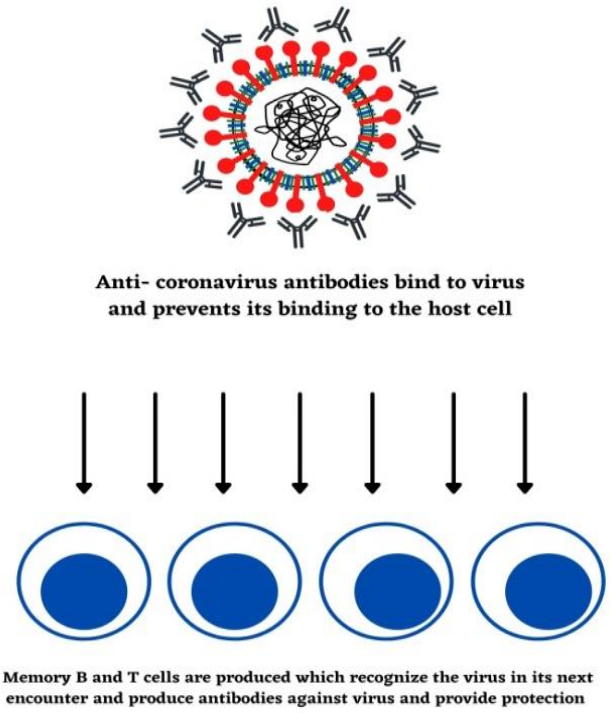


Figure 4 - Basis for development of vaccination against SARS-CoV-2- The S protein of SARS-CoV-2 binds with ACE2/TMPRSS2 receptors on host cell like nasal ciliated epithelial cells or goblet cells, alveolar macrophages etc. The virus is taken up by cell and a vesicle is formed. Viral mRNA is released and translated to protein leading to formation of viral assembly. Replicated virus is released out of cell without lysis of infected host cell.10,13The released virus is recognized by APCs **(**Antigen Presenting Cells**)** like Dendritic cells which engulf the virus and process and present it to cells of adaptive immunity. Virus engulfed DCs express MHC Class I and II **(**Major Histocompatibility Complex**)** and present it to Tcells. T helper cells **(**CD4+ Cells**)** express receptors that bind to MHC Class II. Such activation of T helper cells produces proinflammatory cytokines which in turn activates T and B Cells. Activated B cell proliferates to form Plasma cells which produces antibodies specific to SARS-CoV-2. These antibodies bind to virus and prevents its binding to host cell. The MHC Class I is bound by Cytotoxic T Cells **(**CD8+ Cells**)** that also produces proinflammatory cytokines, activating the immune cell. Cytotoxic T Cells destroy cells infected with virus and provides protection. Memory B and T cells are formed which are long lived and are capable of recognizing virus on its next encounter and produce antibodies **(**17,18, 22**)**.

The most common mode of COVID19 transmission is air – it can either be due to close contact with large droplets or by small droplets called aerosols. The commonest and easiest pathway of entry of SARS-CoV-2 virus into the body is nose. The Intranasal vaccines are administered via the nose rather than injected into the skin **(**10,13,25**)**.

It is well known that following intranasal vaccination to SARS-CoV-2, virus specific TRM cells develop in lungs as opposed to subcutaneous vaccine administration wherein virus specific cells accumulate in spleen25. Indeed, the nasal immunization route may promote the development and accumulation of SARS-CoV-2 specific CD4 and CD8 T cells with a tissue-resident memory phenotype **(**TRM**)** in the upper airway. TRM cells are specialized memory lymphocytes that reside in originally infected tissues instead of recirculating into the blood stream and therefore are capable of inducing a rapid response in case of secondary infection by the same pathogen(17,25) **(**Figure 5**)**.

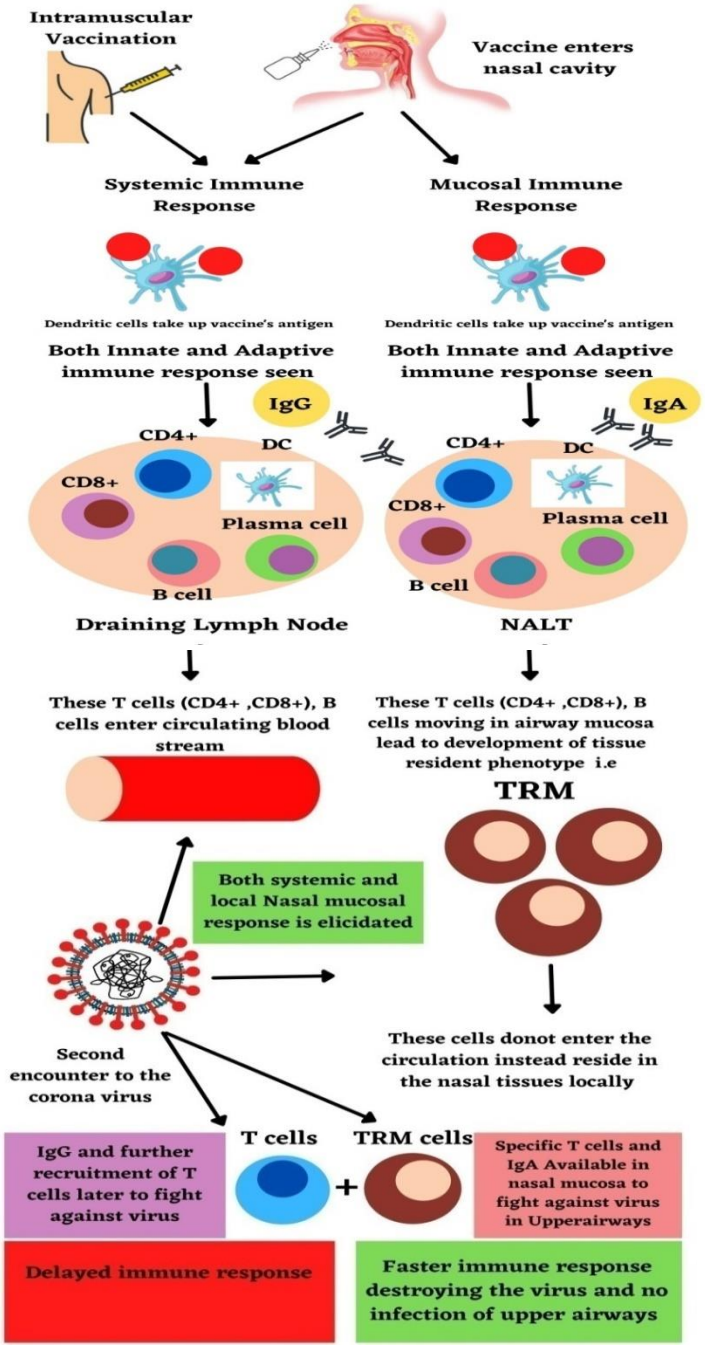


Figure 5 – Comparison of Mechanism of action of Intramuscular and Intranasal COVID19 vaccination. - As vaccine enters the nasal cavity, it is recognized by host as antigen. Cells of innate immune response like dendritic cells capture antigen and get activated. Number of signal transduction pathways are triggered along with activation of cells of Adaptive immune response eg – T helper and Cytotoxic T cells. These T cells present in upper airway mucosa/ NALT develop tissue resident phenotype called TRM **(**tissue resident memory T cell**)**17, 29. TRM are specialized T cells which do not enter circulation, instead reside locally in nasal tissues. This makes TRM cells initiate faster immune response as they are readily available in nasal mucosa to fight against virus during its subsequent encounter. Such a response cannot be seen in case of intramuscular/ parenteral vaccine administration 25, 29. Here TRM cells are not produced and T Cells from draining lymph node enter circulating blood stream. IgA antibodies are produced from plasma cells in NALT with intranasal vaccination as opposed to IgG antibody production by plasma cells from draining lymph nodes on parenteral administration **(**13**)**.

By conducting the vaccination in this way, the vaccine targets the virus present in the nasal mucosa, where the SARS-CoV-2 viral load is likely to be highest. An intranasal vaccine could provide promising results by locally reducing viral load. Such nasal vaccine can be given via a spray, a syringe with no needle or nose drops**(**10,13, 25**)**.

Animal Studies on Intranasal Vaccination against SARS-CoV-2

A study on mice administered with intranasal vaccination with Venezuelanequine encephalitis replicons **(**VRP**)** encoding a SARS-CoV-2 CD4+T cell epitope, induced memory CD4+T cells that are airway CoV-specific and efficiently protected mice against lethal disease through rapid local IFN-gamma production. Airway memory CD4+ T cells differed from lung-derived cells both phenotypically and functionally. As these cells are the first to encounter the viral antigen, they played a crucial role in protection of host **(**mice**)** from disease manifestation. Such kind of protection was conferred by interferon- gamma and early induced innate host response and CD8+ T cells that were specific to virus **(**25**)**.

When an intramuscular vaccination ChAdOx1 nCoV-19 was provided to rhesus macaques, it did reduce the incidence of pneumonia, but did not elicit upper airway mucosal response, and hence did not reduce the viral shedding and thus disease transmission in vaccinated animals. The vaccinated Rhesus macaques showed reduced viral shedding in bronchoalveolar lavage fluid and lower respiratory tract tissues compared to that of control animals. The immune response was majorly mediated by type -1 T helper cells, although a balanced innate and adaptive immune response of type 1 and type 2 T cells was noticed. It was observed that a change in route of administration of vaccination by exposing the respiratory mucosal surface to vaccine, may be crucial in eliciting mucosal immune response, which

might reduce the nasal /upper airway mediated viral shedding thereby reducing the rate of disease transmission **(**26**)**.

A group of researchers transduced replication-defective adenoviruses encoding human ACE2 via intranasal administration into BALB/c mice and established receptor expression in lung tissues. The SARS-CoV-2 infection was manifested in mice that were transduced with hACE2, resulting in higher viral titers in lungs, leading to lung pathology and weight loss. Once the monoclonal antibodies were passively transferred into the mice, fall in viral burden in lungs and mitigated inflammation and weight loss was observed **(**27**)**.

The intranasal or intramuscular immunization of ChAd-SARS-CoV-2, a chimpanzee adenoviral vaccine encoding stabilized spike protein, prevented SARS-CoV-2 lung infection and pneumonia in mice. In particular the Intranasal vaccine delivery generated robust mucosal B and T cell responses along with prevention of upper and lower respiratory tract infection. Thus, intranasal vaccination had highest potential in protecting the animal from SARS-CoV-2 infection and could also promote “sterilizing” immunity that can block interhuman transmission **(**28**)**.

The intranasal vaccine administration generated memory CD4+ T cells that were localized to the airway and were more protective against challenge with pathogenic human coronaviruses than those generated after systemic vaccination. The combination of memory CD4+ T-cell-inducing vaccines with those able to elicit strong neutralizing antibody responses and memory CD8+ T cells would be predicted to result in long-lasting, broad protection against several CoVs. This strategy might also be useful in targeting other pathogenic respiratory viruses. These results indicate that induction of airway memoryCD4+T cells should be considered as a component of any universal human coronavirus vaccine and potentially, those targeting other respiratory viruses **(**25**)**.

Human Intranasal COVID19 Vaccination Updates

Bharat Biotech International Limited - BBV154 Intranasal Vaccine

Bharat Biotech released the results from phase 2 human clinical trials of its COVID19 vaccine, Covaxin in India and after that they began testing an intranasal vaccine in different parts of the country. Hyderabad-based Bharat Biotech, in collaboration with Precision Virologics, a startup incubated at the Washington University School of Medicine in St Louis, US, is working on the first intranasal vaccine for COVID19 in India. Bharat Biotech's nasal vaccine is an adenovirus vector vaccine, made from a weakened version of the common cold virus that was originally sourced from chimpanzees.

The intranasal vaccine is called BBV154, and is being tested to prevent infection and transmission of COVID-19. If the vaccinated individual gets infected, the vaccine is also meant to prevent the progression of COVID-19. Bharat Biotech's other COVID-19 vaccine, Covaxin was found 80.4 percent effective in preventing COVID19. The nasal spray targets and attacks the virus locally **(**in the nose**)** and provokes the immune system to produce antibodies that can combat an infectious organism **(**https://clinicaltrials.gov/ct2/show/NCT04751682 **)**.

Altimmune – Ad COVID Intranasal vaccine

A US company called Altimmune is developing a nasal spray vaccine called AdCOVID. The commencement of phase I clinical trial of AdCOVID nasal spray vaccine was from February 2021. This vaccine is designed in such a way that it stimulates both systemic immunity **(**neutralizing IgG**)** and local immunity **(**mucosal IgA, T cells**)** in the nasal cavity and respiratory tract. Recent Studies have already shown that mucosal immune response is crucial in blocking SARS-CoV-2 viral replication in nasal mucosa, as nasal mucosa may become reservoir for the virus. This will increase the susceptibility of patient to reinfection and disease transmission. Altimmune recently announced the results of AdCOVID nasal spray vaccine trials on animal models. The three critical aspects of the immune system namely serum neutralizing activity, T cell immunity and mucosal immunity were shown to be strongly activated by this single dose intranasal COVID19 vaccine. The company states that vaccine will effectively prevent COVID-19 transmission, especially in children. The trials on a group of 180 volunteers with ages ranging from 18-55 years old, have already been started**(**30**)**.

Codagenix and Serum Institute of India – COVI -VAC nasal vaccine

The UK's Codagenix and Serum Institute of India have developed a nasal vaccine named COVI-VAC, which is a single-dose intranasal, live attenuated vaccine against SARS-CoV-2. The animal studies conducted on this vaccine showed it to be safe and efficacious. COVI-VAC was developed with Codagenix's Synthetic Attenuated Virus Engineering **(**SAVE**)** platform that uses synthetic biology to re-code the genes of viruses into safe and stable vaccines. COVI-VAC has entered Phase 1 clinical trial in first week of January 2021, which is designed as randomized, double-blind, placebo-controlled, dose-escalation trial and 48 volunteers are being tested upon. The primary objective is to evaluate the safety and tolerability of a single dose of COVI-VAC administered by nose drops. The secondary objective of the study is to evaluate immunogenicity, or the vaccine's ability to provoke an immune response, measured as neutralizing antibody, mucosal IgA and cellular immune responses **(**https://www.seruminstitute.com/news.php**)**.

Beijing Wantai Biological Pharmacy Enterprise along with Xiamen University and Hong Kong University – Nasal spray vaccine.

A Phase I clinical trial of an intranasal COVID19 vaccine spray had been approved in China to be started in November 2020. A total of 100 volunteers were enrolled in Phase 1 and is expected to be completed in an year. The vaccine is co-developed by Beijing Wantai Biological Pharmacy Enterprise with researchers from Xiamen University and Hong Kong University. This intranasal spray, consists of weakened flu viruses such as H1N1, H3N2 and B with genetic segments of COVID-19's Spike **(**S**)** protein, that mimics the infection of respiratory viruses and stimulates the immune response. **(**https://www.europeanpharmaceuticalreview.com/news/129563/phase-i-trial-of-intranasal-covid-19-vaccine-spray-approved-in-china/**)**

Conclusion

COVID19 has created havoc globally and till date there is spike in the new cases and increase in the death rates. Vaccines have been developed worldwide to control the pandemic. Most of these vaccines are delivered intramuscularly and are painful and always need assistance. The intramuscular route of vaccination efficiently triggers systemic immune response but fails to activate mucosal immune response. The intranasal vaccines are capable of activating both systemic as well as mucosal immune response. The SARS-CoV-2 enters through mucosal route like nasal, oral and genital, employing a mucosal route for vaccination would be more beneficial to stop the entry of virus. Nasal delivery is the most easily administered route of vaccine. There are certain advantages of this route like easy delivery, painless and not technique sensitive. Nasal vaccine can be self-administered, reducing the risk of infection to healthcare professional. Animal studies have been done on the nasal route of administration and they have showed positive result by locally reducing the viral load. Human trials have also been started by varies companies. The nasal vaccines alter mucosal environment hindering viral entry and replication, which may give promising results in controlling the spread of COVID19 in the future.

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