Review Article

INTRANASAL COVID VACCINES FOR SARS-CoV-2

Abstract

As the nasal mucosa is generally the first site of infection, intranasal vaccinations have a distinct advantage over conventional COVID-19 vaccines. According to preclinical and clinical investigations, intranasal vaccination results in significant neutralizing antibody production and mucosal IgA and T cell responses, which prevent SARS-CoV-2 infection in both the upper and lower respiratory tracts. The nasal formulations are non-invasive and have a lot of patient appeal. Intranasal vaccinations allow for self-administration and may be made to persist at room temperature, reducing transportation and storage logistics. We give an overview of nasal vaccinations in this review, with an emphasis on formulation development and ongoing preclinical studies and clinical investigations for SARS-CoV-2 intranasal vaccine preparations.

Keywords

COVID-19, SARS-CoV-2, Nasal vaccine, Nasal spray, Antigen-presenting cells (APCs), Dendritic cells, NALT- nasopharynx-associated lymphoid tissue, MALT- mucosa-associated lymphoid tissue, BALT- bronchus-associated lymphoid tissue.

Comment [Review1]: Better to read as: Nasopharynx associatedlymphoid tissue (NALT), mucosa associated lymphoid tissue (MALT), bronchus-associated lymphoid tissue (BALT).

1.Introduction

Vaccines use the immune systems of humans, amazing ability to respond to it and remember harmful material it deals with. An ideal vaccination would prevent severe disease, hospitalization, and death by providing quick, multifaceted, and longterm protection. This adaptive immunity is mediated by B cells that produce antibodies and T cells after vaccination. COVID19 vaccine has been delivered to a bout 4.3 billion people in about 180 countries at a rate of 42.5 million doses per day. More than 180 potential vaccines against SARS-Cov-2 were in various phases of development as of May 2, 2021, including early-stage and clinical development.[1] The intramuscular injection causes a robust serum IgG response, which is thought to protect the lower respiratory tract, but not the epithelial cell IgA responses, which are thought to protect the upper respiratory tract. Through the muco ciliary process, IgA can reach the upper respiratory tract, but only if the serum IgG content is high.[2] As a result, most of the vaccines only protect against diseases of the lower respiratory tract and do not induce sterilizing immunity in the up per part. Nasal vaccine delivery not only protects against clinical disorders but also has an additional function to stop virus spread among affected people.[3]

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For preventing virus transmission, a vaccine that induces sterilizing immunity in the upper airway will be desirableInt ranasal vaccination is a promising approach since it closely mimics the typical route of infection, is simple to administ er, and has the potential to obtain a significant market share in the future. Intranasal immunization showed strong n eutralizing antibody responses as well as mucosal IgA and T cell responses, nearly eliminating SARSCoV2 infections in the upper and lower respiratory tracts.[3,4]. Unlike injections, a nasal spray is painless and patient convenient.

Comment [Review4]: Separate the two words by putting a fullstop between thewords

Comment [Review5]: Rephrae: convenient to the patient.

2.Lifecycle of COVID 19

A coronavirus is made up of a lipid bilayer wrapped around a glycoprotein surface with spike-like projections. Spike protein, Membrane protein, Envelope protein, Nucleocapsid, and RNA Genome are the five types of proteins that make up its structural component. In addition to the receptor-binding motif, the S protein has two domains or subunits: an S1 subunit which is a lobular receptor-binding domain) and an S2 subunit which is the stalk fusion domain). It is an enclosed virus because it contains non-segmented positive-sense single-stranded RNA that is encapsulated in a capsid made of protein.

The S-protein binds to the Angiotensin-converting enzyme 2 receptors present in the lung epithelia of the host. Endocytosis facilitates viral entrance into the host cell since the virus exploits human cell machinery. Following receptor enga gement by endosomal cysteine protease cathepsins, the virus must enter the cytoplasm of the host cell. [5].S1/S2 cleavage is used by transmembrane protease serine 2 (TMPRRS2) or TMPRSS11D to activate S-protein, which leads to membrane fusion of the virus and cell.

Protein cleavage occurs at two points within the S2 protein element, with the first cleavage separating the Sreceptorbinding protein's domain from its fusion domain, and the second cleavage facilitating membrane fusion.[6] The coronavirus life cycle proceeds to tr anslate the replicase gene from the virion genomic RNA as the nucleocapsid has escaped and become uncoated,

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As its RNA gets translated, formed into the new virus, and discharged by the injured
cells, a variety of proteins are produced. A humoral immune response is launched by the host immune system against invading SA RS-CoV-2 (i.e., antibodies).[7] Immunoglobulins levels rise, signalling the immune system to respond to the infection.
As a result, the host may have respiratory symptoms, which may be followed by a cytokine storm, which results in vasodilation and hypotension. As a result, the volume of blood reaching the organs is limited, resulting in organ damage. The hypothalamus is also a ffected by cytokines like IL-1, IL-6, and TNF-, which causes fever.[8] Considering SARS-potential CoV-2's to mutate and create new forms, developing a completely functional vaccine could be a challenge.
The WHO has classed 'triple mutant' mutants detected in India and California as delta variants.
The US Food and Drug Administration (FDA) had found and classed five variants of concern at the time of writing, whereas eight va riants of interest had been identified and classified. [9]
These new strains, it is feared, would elude both natural infection-induced immunity and existing vaccination measures.[10]

3.Therapeutic potentiality of nasal vaccines

Pfizer/BioNTech and Moderna's mRNA vaccines are accessible in the United States, the United Kingdom, and other countries. Thr ee adenoviral vector vaccines have been approved: Oxford/AstraZeneca AZD1222 (Covishield in India), JNJ-78436735 (Janssen Pharmaceutical, USA), and Gamaleya Sputnik V (Russia).
Sinopharm and Sinovac in China create inactivated coronavirus spore-based vaccines, which are the third type of vaccination. They're normally given intramuscularly in the upper arm in one or two dose s. [11]
Local reactions to intramuscular injections, such as pain or oedema at the injection site, are common.
Intramuscular injections cause a systemic humoral response to a vaccination, which is mediated by B cells and results in the produ ction of IgM antibodies initially, then IgG antibodies.[12]
Humoral immune systems are specialized by high amounts of primary IgM antibodies, as well as secondary IgG, IgA, and IgE antib ody responses, all of which are consistent with acquired immunity.
This process works in tandem with the immunological response of T cells.
In respiratory viruses , however, the mucosal immune system is the first line of defence in the nasopharynx-associated lymphoid tissue (NALT), with pathogen-induced reactions primarily mediated by IgA antibodies produced by mucosal epithelial cells.[13]
These structures in the upper airways include the palatine tonsils and other lymph epithelial complexes in Waldeyer's pharyngeal ri ng, such as the adenoids in mammals.
In COVID-19 infection, IgA and IgG responses exhibit a negative relationship; that is, powerful systemic IgG response to vaccination cannot be accompanied by an adequate mucosal IgA response.[14]
Because of a lack of mucosal protection, systemically vaccinated persons are susceptible to SARS-CoV-2 infection through the upper respiratory tract if they are asymptomatic. Lymphatic tissues, specifically mucosa-associated lymphoid tissue (MALT), are located in mucosal tissue of the nose, lungs, gastrointestinal system, and vaginal/rectal sur faces, and are linked to mucosal immunological activity. It is further divided into nasal-associated lymphoid tissue (NALT) and bronchus-associated lymphoid tissue (BALT) in the lower respiratory tract. Epithelial cells, lymphocytes, and underlying antigen-presenting cells, cytokines, and chemokines mount an endogenous, nonspecific, and adaptive immune response in response to pat hogenic organisms or immunogenic substances.[15] Epithelial cells can recognize and take up pathogenic species and/or antigenic components via nonspecific endocytosis or communication with pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs). NALT is made up of lymphoid tissue, B cells, T cells, and APCs, and is surrounded by an epithelial layer of m emory (M) cells (antigen transporter cells).
Pathogens and toxins are prevented from sticking to or infecting epithelial cells and damaging the mucosal barrier when antigen-specific secretory IgA (sIgA) antibodies are activated. Pathogens or immunogenic substances may be able to communicate with AP Cs, such as macrophages and DCs, through the nasal epithelium.[16] Before travelling to the lymph node, where the immunogenic component is delivered to T cells to initiate the immunological cascade, APCs filter the antigen. Soluble antigens are recognized by APCs, but particulate antigens are generally picked up by M cells and delivered to NALT by M cells. NALT drains to the lymph node , where it is used to produce more antigen. IgA secretion can occur as a result of
antibody response with pathogens or antigensIntracellular antigens are typically kept in host cells before being coupled to a cell sur face protein, the major histocompatibility complex I (MHC-I), and transported to the cell surface.[17]CD8 + T cells become cytotoxic T lymphocytes when MHC-I is present on their surface (CTLs). MHC-II molecules offer endocytosed extracellular antigens for activation.Th17 CD4 + cells may also be stimulated by a nasal vaccination. Proinflammatory interleukins such as IL-17A, IL-22, IL-17F, and IL-21 are produced by Th-17 cells. As a result, efficient vaccines that protect these locations are urgently required. Memory T cells that are linked to CD8+ T cells have a long lifespan in terms of systemic immunity. Apart from the convenience of administration, an intranasal vaccine's muc osal IgA response should provide effective systemic protection.[18]. Antigen activated B and T cells leave the draining lymph nodes after mucosal immunization, travel across the lymph, enter the circulation, a nd seed the mucosal tissues. Adjuvants gave intranasally increase immune responses by enhancing the innate immune response by upregulating the expression of costimulatory molecules, chemokines, and cytokines.[19] Crossreactive antibodies have been demonstrated to be activated by intranasal immun ization, which could indicate cross-protection. Because cross-protective vaccines can cause cross-reactive antibodies to form that recognize more than one antigen, this effect could make vaccines more effective by lowering the nu mber of immunizations required.

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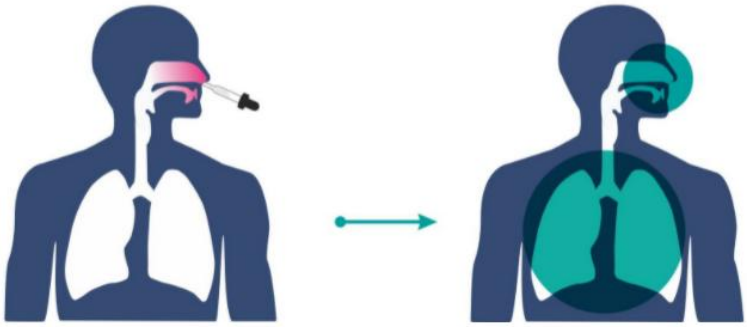
Comment [Review9]: Delete “that” and use “and” instead

Fig 1: Human model for infection prevention



- Likely to prevent infection and transmission
- Likely to prevent disease

4.Mucosal immune system

The mucosal immune system, also known as mucosa-associated lymphoid tissue (MALT), which is found in the mucosal tissues of the nose, lungs, gastrointestinal tract, vagina, and rectum, should ideally protect humans from pathogens entering the body through mucosal membranes.[20] The MALT includes proximal structures such as the nasopharynx-associated lymphoid tissue (NALT), bronchus-associated lymphoid tissue (BALT), and gut-associated lymphoid tissue (GALT), depending on their location.
As a result, mucosal immunity is frequently best produced by administering vaccines through the mucosal route, because mucosal immunization will generally result in both a mucosal and a systemic immune response provided an adequate vaccine formulation is developed.
[The nasal and oral routes of mucosal administration are the most acceptable and accessible, but the nasal route is preferred over the oral due to the hostile gastrointestinal environment, where the antigen can potentially be degraded or denatured, and the dilution by intestinal content, which necessitates high doses of antigenic material and specialized vaccine formulations.[20]

5.Mucosal Immune response

Systemic IgG antibodies, T cell responses, and mucosal antibody responses in the form of secretory immunoglobulin A are all induced by a natural respiratory viral infection.
[21]The upper respiratory tract, such as the nasal cavity, is thought to be mostly protected by SIgA, while the lower respiratory tract is primarily protected by IgG. The IM vaccination suppresses systemic virus multiplication but only provides minimal mucosal protection via IgG transudation to airway surfaces, such as the lungs. It is widely assumed that, whereas mucosal vaccination produces large levels of protective secretory IgA antibodies at the mucosal site but low levels of systemic IgG antibodies and cell-mediated immunity, parenteral vaccination produces the opposite.[22]

Matsuda et al. (2021) also claim that there are numerous examples of IM non-replicating vaccines failing to protect against respiratory virus infections, such as RSV, parainfluenza virus type 3, Ad4, rotavirus, and measles vaccines. IM vaccinations against respiratory viruses may generate disease-preventing or disease-attenuating immunity but not "sterilizing" immunity.[23]

Comment [Review10]: Write in full, then abbreviation (IM))

A DNA vaccine expressing the fusion gene of the bovine respiratory syncytial virus was delivered IM to calves and generated antigen-specific IgG and IgA responses in sera and BAL fluids, resulting in a substantial protection against a pathogen challenge. [24]However, the protection against BRSV infection was not as strong as it had been after a previous infection. In the case of influenza vaccines, either IN (30 g) or IM (2 10 g) inactivated influenza virus vaccines elicited antibody-secreting cells in the bone marrow and memory B cell dispersion to organized lymphoid tissue; however, the IgG response was strongest after IM injection, whereas IgA production was only evident after IN vaccination.
After the IM vaccination, the authors hypothesized that broad dispersion of IgG memory B cells to secondary lymphoid organs, such as Peyer's patches and the NALT, would ensure quick activation in the event of influenza infection.[25]

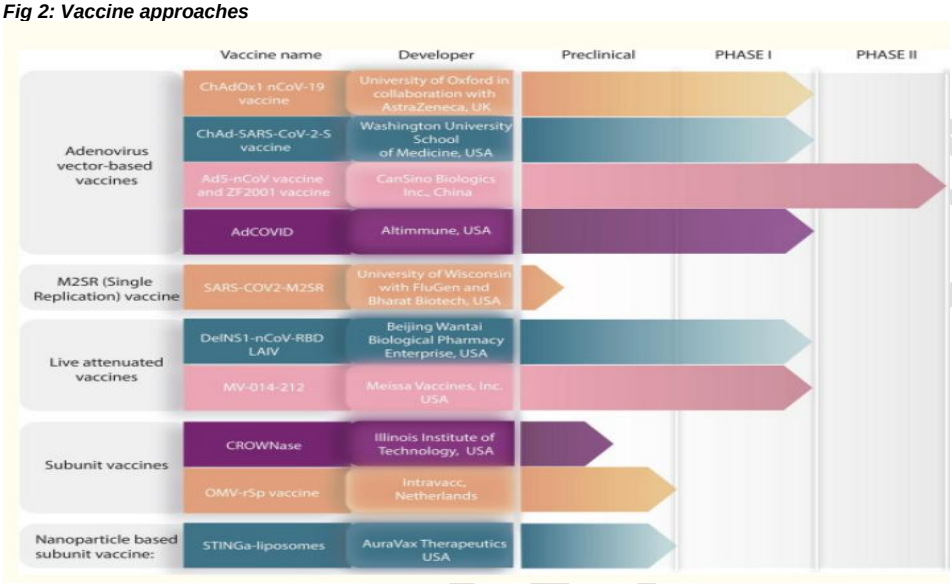
Rabbits were immunized with an HPV 6bL1 DNA vaccine against human papillomavirus via IM and vaginal administration in another study. For up to 14 weeks following vaccination, the mucosal delivery generated 6bL1 virus-specific IgA antibodies in the vaginal secretions, which showed neutralizing activity in a hemagglutination experiment.
After IM immunization, no mucosal immune response was identified in vaginal secretions.[26]

In addition, a study compared the immunological effects of a novel inactivated entire trivalent influenza virus vaccine given IN as a prime/boost vaccine 21 days apart in 21 elderly people to a single dose of a commercial IM influenza vaccine given to 22 elderly subjects[27]. A hemagglutination inhibition test and an ELISA were used to assess serum IgG and IgM antibodies, as well as nasal IgA. The mucosal IgA response was found to be 47.6–71.4 per cent and 18.1–31.8 per

cent for participants given IN and IM vaccinations, respectively, but the observed serum antibody response was found to be 20.0–61.9 per cent and 18.2–72.7 percent for the two modes of administration, respectively. At the end of the trial, 57.1, 65.0, and 50.0 percent of the IN vaccinated subsets were seroprotected against A/Beijin, A/Sydney, and B/Harbin, respectively, and 68.1, 77.2, and 54.5 per cent of the IM vaccinated subsets were immune to A/Beijin, A/Sydney, and B/Harbin, respectively.[30] The scientists concluded that the IN vaccine was much more efficient than the IM vaccine in eliciting a mucosal IgA response, which they said could help prevent influenza in its early stages and reduce morbidity and complications in the elderly.[31]

6.Vaccine approaches

Fig 2: Vaccine approaches



SARS COV2 antigen selection[32]

To mediate viral entry in the upper and lower respiratory tracts, the SARS-CoV-2 S protein interacts largely with ACE2 receptors. On the virion's surface, the mature S protein is a trimeric class I fusion protein. The S1 segment contains the receptor-binding domain (RBD), while the S2 fragment contains the fusion peptide. Infected humans develop powerful neutralizing antibodies against the S protein, particularly the S1 fragment with the SARS-CoV-2 receptor-binding domain (RBD), according to various monoclonal antibody investigations.
The N protein was tested for efficacy in early SARS-CoV-2 vaccination experiments, however, in vivo models revealed that N-based vaccines provided no protection. Furthermore, increased pulmonary eosinophilic infiltration resulted in a worsening of the illness. Due to their lesser immunogenicity, M and E proteins are less appealing as vaccine targets.

- *6.1 Live attenuated vaccines[33]*

Live attenuated vaccines are made from pathogenic viruses that retain their capacity to infect and multiply cells but have been treated to cause no or very minor sickness. Growing the virus under unfavorable conditions, such as at a low temperature, or rational change of the virus genome optimization removal of genes that prevent innate immune recognition can complete attenuation.
These procedures, however, are timeconsuming and technically tough, resulting in a difficult and lengthy development process. After a prime/boost vaccination schedule, a live attenuated virus, which is almost identical to the wild virus that causes infection, usually produces a powerful and long-lasting humoral and cell-mediated immune response.

Furthermore, because the virus continues to replicate after vaccination, the immune response targets both structural and nonstructural viral proteins, broadening humoral and cellular immune responses without the addition of adjuvants, as these vaccines already include naturally occurring adjuvants.
This type of vaccines, such as the quadrivalent influenza vaccine against A(H1N1), A(H3N2), and two influenza B viruses available on the market under the trade name FluMist Quadrivalent, can be given intranasally to produce a mucosal immune response. It comes as a 0.2 mL suspension in a single-dose pre-filled intranasal spray device that should be divided in half and sprayed into each nostril.

- *6.2 Inactivated viral vaccines[34]*

The entire disease-causing virus or a portion of it is frequently included in inactivated viral vaccinations. As with coronaviruses (e.g. SARS-CoV-2), immune responses are likely to target a variety of proteins, including the S but also M, E, and N.

When compared to live attenuated vaccines, inactivated vaccines only stimulate antibody-mediated responses, which can be weaker and shorter-lived. As a result, inactivated vaccines are frequently given with adjuvants, and booster doses may be required. Biosafety level 3 facilities are required for vaccine manufacture, in which the virus is cultured in cell culture (typically Vero cells) before being inactivated.
The virus's productivity in cell culture may have an impact on the final product yield.
This vaccine is both safe and effective in the protection of diseases such as polio and influenza.

- *6.3 Recombinant vector vaccine[35]*

Vaccines based on viral vectors use a modified virus to transfer the genetic code for an antigen (e.g., the S protein in the case of COVID-19) into human cells, which subsequently manufacture the antigen.
This form of vaccine imitates a genuine viral infection by infecting cells and training them to produce the antigen to elicit the desired immune response.T cells respond with a powerful cellular immunological response, and B cells produce antibodies as a result of this mechanism. The viral vectors are grown in cell lines, and they are simple to make.
There are two types of viral vectors: replicating and nonreplicating. Replicating viral vectors can replicate and hence can manufacture new viral particles, ensuring a steady supply of vaccination antigens for lengthy periods. When compared to nonreplicating viral vectors, this results in a higher immune response with a single dosage. Replicating viral vectors are chosen so that the virus does not infect the host and cause sickness. They're usually made from attenuated viruses that have been genetically modified to express a specific antigen protein, such as the S protein in the COVID-19 vaccine.
Nonreplicating viral vectors, on the other hand, do not retain the potential to produce new viral particles since the crucial viral genes for replication have been deleted. The most popular methods for administering this vaccination are via intramuscular injection of adenovirus. The utilization of live pathogen viruses is not required in the manufacturing of viral vector vaccines, and the vectors may be cheaply made in large quantities, demonstrating good activation of both B and T cell responses in-vivo.
The vaccine's efficacy can be neutralized by pre-existing vector immunity, which is a disadvantage. However, utilizing vectors that are uncommon in humans originating from animals, or viruses that do not create much immunity, this problem can be readily avoided.
Furthermore, because vector immunity can be an issue during the second dosage of a prime-boost regimen, using two separate viral vectors for the two doses can help avoid this issue. Nonetheless, vaccine antigen can only be produced in this situation for as long as the initial vaccination is present in infected cells, resulting in a generally weaker immune response. Booster dosages will very certainly be required.

- *6.5 Protein subunit vaccines*

Protein subunit vaccines comprise pure antigenic pieces such as isolated proteins that have been chosen for their ability to stimulate the immune system.
Antigens such as specific isolated proteins from viral or bacterial pathogens, chains of sugar molecules found in the cell walls of some bacteria, or a carrier protein binding a polysaccharide chain to stimulate the immune response can all be used to make acellular vaccines. Acellular vaccinations are typically thought to be extremely safe because they do not cause disease.
Booster doses are frequently required because the immunological response is not as strong as with live attenuated vaccinations. One potential drawback of this form of the vaccine is that isolated proteins may be denatured, causing them to attach to antibodies that aren't specific to the pathogen's protein.
The antigenic proteins employed in SARS-CoV-2 are the S protein and the RBD. This sort of vaccine has the advantage of avoiding the handling of live viruses. Acellular pertussis (aP) vaccines, which include inactivated pertussis toxin detoxified either by chemical treatment or by molecular genetic procedures, are widely used protein subunit vaccines.
Alum is used as an adjuvant to boost the vaccine's efficiency by promoting a greater antibody response.
Allen and Mills (2014). Another acellular vaccination against Hepatitis B uses recombinant technology to manufacture the hepatitis B virus surface antigen. Even the adjuvant aluminum phosphate or aluminum hydroxide is used in this vaccination to increase the immune response once it is given.

- *6.6 RNA and DNA vaccines*

In comparison to previous vaccines, nucleic acid-based vaccines use distinct strategies.
Rather than directly delivering the protein antigen to the body, they transfer the antigen's genetic code to the body's cells, instructing the cells to manufacture the antigen, which will later trigger an immune response. These vaccines are the most promising vaccines for the future since they are rapid and easy to develop.
RNA-based vaccinations and DNA-based vaccines are the two types. RNA vaccines are made up of messenger RNA or self-replicating RNA that is usually packaged in a particulate carrier like a lipidic bilayer membrane.
When mRNA first enters the body, this formulation shields it and aids cell internalization.
For mRNA, higher doses are necessary than for self-replicating, self-amplifying RNA. When the mRNA is inside the cells, ribosomes can translate it into the antigen protein, which triggers the immunological response. The body then spontaneously breaks down and eliminates the mRNA.The vaccine can be made completely without the use of cell cultures, but long-term storage stability is difficult because it requires frozen storage.
Because RNA-based vaccines are often given by injection, they are unlikely to induce substantial mucosal protection.DNA does not need to be formed in particle carriers because it is more stable than mRNA/RNA.
They're made from plasmid DNA, which can be made in vast quantities in bacteria. Mammalian expression promoters and the specific gene that encodes for the antigen produced following uptake in the vaccinated person's cells are found in the DNA.

They frequently require delivery tactics such as electroporation to aid DNA cellular absorption to be administered. Both of these nuc leic acid-based technologies are at the
edge of vaccination, with two different mRNA vaccines approved for human use and the most advanced DNA vaccine so far being I novio's INO-4800, which has entered Phase 2 and 3 clinical trials.

7.Challenges

Mucosal immunity gives efficient and prolonged protection against coronavirus infection since SARS-CoV-2 infection occurs on the mucosa of the respiratory system. Anyhow, all commercially available emergency vaccinations are administered intravenously, resulting in only humoral immune responses and no CoV-specific mucosal protection. Various parameters, including antigen, adjuvant, formulation, and animal models for effectiveness and safety evaluation, should be considered for successful and safe nasal vaccine manufacturing. Antigens for responding to a specific adaptive immune response; immunostimulants to stimulate the innate immune system; and a mechanism for vaccine delivery are usually included in successful nasal vaccine development.
One of the most difficult components of creating a nasal vaccine is nasal clearance. Mucus works as a sticky solvent in the nasal mucosa. Inhaled air travels through Cilia, a protective barrier that prevents harmful chemicals, germs, and debris from enter ing the lungs. The amount of time an antigen spends in the mucosa affects how well it is absorbed. As the mucosal clearance increases, antigen absorption decreases. A vaccine delivered intranasally is more likely to trigger Th17 immune responses, making it mor e difficult to move SARS-CoV-2 out of the lungs. A demand for a specialized delivery mechanism, which can add to the vaccine's cost, is another stumbling hurdle to a nasal COVID-19 vaccination.
A good vaccine formulation for intranasal administration, with or without addi tional adjuvants, maintains the antigen stable, ensures it stay in the nasopharyngeal region long enough to interact with the lymphatic system, and activates the immune system to provide long-term protection.

8.General advantages and disadvantages

Human viruses like influenza and SARS-CoV-2 invade the body through the respiratory system, thus it's the only basis to investigate and evaluate the possibility of formulating nasal vaccinations to treat these diseases. Nasal vaccines are an alternative to parenteral vaccines because of the possibility of dose lowering than the injection and is a site targeted, namely the NALT, nasal formulation do not require the assistance of professionals, and it is a better option for children who dislike injections in genera l. Furthermore, nasal vaccines may be given utilizing simple nasal devices, so it does not require an aseptic environment for administration, which is very beneficial for immunization programs in impoverished countries. There have also been dry powder nasal immunizations developed, which avoid the requirement for cold-chain manufacture and save money.

Follicle-associated lymphoid tissue, or NALT, is encased by the nasal epithelium and is important for producing mucosal immune responses, particularly at Waldeyer's ring in the nasopharynx. Nasal vaccines induce both humoral and cell-mediated immune responses, as well as serum IgG and local nasal neutralizing mucosal IgA, all of which protect against invading pathogen colonization, as discussed more below. Intranasal vaccination has also been demonstrated to produce cross-reactive antibodies, suggesting cross-protection. After IM/SC vaccine administration, systemic viral replication stops, but only a small amount of mucosal protection in the form of IgG transudation to airway surfaces is elicited, as detailed further below.
As immune responses depend on the type of vaccine formulation, it's difficult to select the best delivery system for the nasal vaccine. Furthermore, for the vaccine to reach the NALT, it must stay in the nasal cavity/nasopharynx for an extended period. This is commonly done with bioadhesive liquid or powder vaccine formulations that can partially bypass the mucociliary clearance process. The potential problem of nasal vaccinations having toxicological consequences will be examined further down.

9.Vaccines in development

9.1 Alt-immune Inc.[36]

AdCovidTM, a single-dose COVID-19 vaccine based on a replication-deficient (Ad5)-vectored vaccine encoding for the receptor-binding domain (RBD) of the SARS-CoV-
2 spike (S) protein, is being developed by Altimmune Inc. In a preclinical investigation in mice, the immunogenicity of AdCOVIDTM was assessed after intranasal delivery of one of three vaccination doses: 3.35 108 ifu (high-dose), 6 107 ifu (mid-
dose), or 6 106 ifu (low-dose) in a volume of 50 L, or buffer control.
The vaccine showed significant mucosal immunity, with a 29fold rise in mucosal IgA in the respiratory tract as evaluated in BAL flui d, and strong IgG serum neutralizing activity, many times greater than the FDA-recommended titer. Furthermore, antigen-specific CD8 + killer T cells were identified in the lungs as early as 10 days after vaccination, indicating a powerful activation of cell-mediated immunity. There were no nasal samples taken to identify secretory nasal IgA. The researchers found that their AdCOVID TM vaccination elicited humoral and cellular responses at both systemic and mucosal locations, particularly in the lungs, which are a common site for infection and illness.
In Phase 1 clinical trial, up to 180 healthy adult volunteers between the ages of 18 and 55 will be evaluated for the safety and imm unogenicity of a single dosage of AdCOVIDTM.
AdCOVIDTM will be given to subjects as a nasal spray in one of three dosing levels. In addition to the primary research endpoint, s erum IgG binding and neutralizing antibody titers, mucosal IgA antibody levels from nasal samples, and T cell responses will also b e used to assess AdCOVIDTM's immunogenicity. On February 25th, 2021, the FDA approved the research.

9.2 Codagenix Inc.[37]

Codagenix Inc. has developed an intranasal vaccine against SARS-CoV-2 (COVI-
VAC), which is a live attenuated entire viral platform that employs "synthetic biology" to re-
code virus genes into a safe and stable vaccination. The "de-optimized" COVI-

VAC virus from Codagenix can be easily produced in cell culture. Results from preclinical research have not been published as far as the present authors are aware, and the only information accessible is from a news review.
In the UK, however, a phase 1 clinical trial is
underway to assess the safety and immunological responses of intranasally delivered COVI-VAC in healthy young participants.
The subjects will be randomly assigned to one of three groups: two doses of COVI-
VAC, 28 days apart, two doses of placebo, or one dosage of COVI-VAC and one dose of placebo.
Drops are inserted into each nostril to deliver the dose. For 14 days, each subject will keep track of any symptoms and mouth temp erature. To test the immunological response, blood samples and intranasal samples will be taken. On December 22nd, 2020, the M HRA accepted the study plan. The primary subject was dosed on January 12th, 2021.

9.3 AstraZeneca[38]

The same vaccine was tested in hamsters and nonhuman primates by AstraZeneca/Oxford Jenner Inst.
The animals were protected against pneumonia after receiving an IntraMuscular injection of the vaccine, but there was no reduction in sub-
genomic and genomic viral shedding (RNA) from the nasal cavity, with shedding similar to that of control animals, indicating that the virus was replicating in the upper respiratory tract.
A single IN dose of ChAdOx1 covid-
19, the same amount of vaccine given IM, or an IM control vaccine was given to three groups of ten Syrian hamsters.
The animals were given 40 L of 104 TCDID50 SARS-Cov-2
human viruses intranasally 28 days after immunization in a challenge study.
Vaccinated animals were housed with nonvaccinated donor animals for a
few hours in a transmission experiment. Both modes of vaccination resulted in high IgG titers with no differences between them.
IN vaccinated animals had considerably stronger neutralizing antibodies.
On days 1–3 and 6–7, viral RNA was identified in nasal swabs from all animals, although it was considerably lower in IN-vaccinated animals compared to controls.
Only 7 days after vaccination did oropharyngeal swabs from IM vaccinated animals show a substantial reduction in viral RNA when compared to controls. In the case of infectious virus, there was a substantial difference in the amount of virus in oropharyngeal swa bs between IN vaccinated and control animals, however, there was no change in the amount of viral RNA or infectious virus betwee n IM vaccinated and control animals.
In addition, no viral RNA or infectious virus was found in the lung tissue of IN-vaccinated animals.
Four rhesus macaques were vaccinated IN with 2.5 1010 virus particles ChAdOx1 of-
19 in a prime/boost regimen and compared to four control animals in the nonhuman primate experiments.
106 SARS Cov2/human virus particles were administered intravenously and nasally to the animals.
In comparison to BAL fluid and serum samples, nose swabs had higher proportions of IgA to total Ig antibodies. At day seven follow ing the prime immunization (49 days post-infection DPI), S and RBD-
specific IgG antibodies were identified in serum and nasal swabs but not in BAL fluid.
Following the booster vaccine, IgG titers were found to be higher (-28 DPI).
SARS-CoV-2 specific IgA
was low after the primary immunization but increased following the booster vaccine, and was also found in BAL fluid 7 days later.
In vaccinated animals, serum neutralizing antibodies were identified
at titers similar to those obtained in prior IM vaccination trials. Control animals' nasal swabs included genomic and subgenomic RN A as well as an
infectious virus after being challenged. Vaccinated animals' nasal swabs contained viral RNA, but at a lower quantity and in fewer a nimals. All control animals' BAL fluid contained genomic and sub-genomic RNA.
At early time points, genomic RNA was identified in all four vaccinated animals, whereas sub-
genomic RNA was only discovered in one animal at low levels.
In vaccinated animals, no infectious virus was found in BAL fluids, and the viral load in the lungs was much lower than in control ani mals.
After IN immunization there was no difference in viral load in the nasal cavity.
As a result of the IN vaccine, there was less shedding and a lower viral load in the BAL fluid and lower respiratory tract tissue.

10.Safety and potency[39]

The growing number of clinical research illustrates the increasing demand for intranasal vaccinations that are easy to give a nd offer formulation benefits over other vaccine routes.
The benefits of directly enhancing the mucosal immune response intranasally are obvious, but they have yet to be completely realized, except those for influenza, which demonstrate the route's effectiveness. However, the urgent need to immunize large populations against COVID-19 has highlighted the importance of having methods in place. Several safety-related occurrences may be noticed only during long-term surveillance for adverse events after immunization due to rarity or etiology. It will be critical to have robust safety follow-up measures in place, both during emergency use and following licensure, to identify any potential uncommon incidents. Because pregnant women and children have not yet been included in clinical trials of SARS-CoV-2 vaccinations at the time of writing, these studies must be done for both efficacy and safety.
There is no data on the safety of RNA-based immunizations in children or during pregnancy because they are a new platform. Adventure-based vaccines (Ad26 and Ad5) have been licensed for Ebola, while there is no significant data on safety during pregnancy. Because the majority of COVID intranasal vaccines are currently in early-stage clinical trials, their safety and efficacy in humans remain uncertain. As a result, statistics on intranasal influenza vaccine safety and efficacy are more reliable.

The licensed intranasal influenza vaccinations FluMist/FluenzTM and NasovacTM live attenuated influenza nasal spray have had no major side effects, suggesting their safety. Naso vac, on the other hand, has significant efficacy data, but Flu Mist is one of the most successful intranasal vaccines.[40]
The effect of vaccines, particularly intranasal sprays, varies by year, patient variables, vaccination type, and even virus kind and subtype. As a result, clinically confirmed effective vaccines must be re-evaluated from time to time in new demographic data sets.

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