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Innovative Therapeutic and Vaccine Approaches Against Respiratory Pathogens

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ABSTRACT

Respiratory infections are a major global concern and are mainly caused by pathogens like bacteria and viruses. Hence, there is a need to treat these infections by using appropriate and effective therapeutics and vaccines. But it has been observed that many pathogens become anti resistant to antibiotics and antivirals. This is a major concern and that is why continuous research is being carried out to develop the available treatment techniques and develop some innovative approaches against these pathogens.

In this report, I will discuss various innovative and developed therapeutics and vaccine technology used against respiratory pathogens. Scientists have come up with some improved techniques like IgY-based and peptide-based therapeutics, which are quite effective and provide better protection than other conventional therapeutics. Apart from this, some vaccines like intranasal DNA vaccines have also proved to be effective than the earlier used vaccines.

INTRODUCTION

Respiratory infections are increasing at an alarming rate and the main cause of these infections are bacteria and viruses. Among all the respiratory pathogens, viruses are the major cause for causing these infections. Some common respiratory pathogens are Respiratory syncytial virus (RSV), influenza viruses, *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*,*Haemophilus influenzae* and many more. Although these bacterial pathogens are less common than viral pathogens, but they still cause some serious infections.

With advancement in vaccine technology, many therapeutic strategies and vaccines are made to treat these infections effectively. Various techniques are being discovered for the detection of these pathogens in early stages. However, during diagnosis multiple pathogens are being detected more often now which increases the risk of infection by multiple pathogens. For this, innovative therapeutic approaches are needed to be taken into consideration which will focus on all these pathogens and helps in treating them effectively.

TYPES OF RESPIRATORY PATHOGENS

Respiratory tract infections are caused by a various viruses and bacteria. Although the symptoms are quite similar but the treatment and diagnosis of the pathogen responsible for the infection is very different. Some common viral respiratory pathogens are Respiratory syncytial virus (RSV), influenza viruses, parainfluenza viruses, and human coronavirus. *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*,and *Haemophilus influenzae*are somecommon bacterial respiratory pathogens in humans. These pathogens are responsible for causing upper and lower respiratory tract infections. The upper respiratory tract hosts a variety of potential pathogens which can overgrow and cause infections under certain conditions.

PATHOGENESIS OF RESPIRATORY PATHOGENS

Pathogenesis is the process by which a pathogen affects our body and causes disease. In order to infect the host, the virus enters the host through mucosal linings of the respiratory tract i.e., nasal canal. After entering the respiratory tract of the host, the virus adheres to specific receptors present on the epithelial cells of the respiratory tract. Various factors like environmental factors (pH, temperature, humidity etc), host-dependent factors (age, immunity etc), virulency, affect the infection process of the virus.

However, virus only affects the susceptible cells that contain functional viral receptors. These viral receptors are the site of adherence of the virus to the target cells. Hence, if there is no such receptor, the virus will not be able to attach itself to the target cell and will eventually does not cause infection. The interaction of the virus to the cell receptor is facilitated by some specific surface proteins present on the virus. Some enveloped viruses like influenza virus can completely fuse with the cell membrane of the host cell and transfer its nucleocapsid into the cytoplasm of the host cell, while some naked viruses can fuse with the host cell by the process of endocytosis.

After its entry into the host cell, the virus tends to complete its life cycle. If it is a lysogenic virus, then it will incorporate its genome to the host genome and will make its viral copies by using the host machinery for replication. But if it has a lytic life cycle then it will first synthesis viral components including the capsid and proteins and then they are assembled in the later phases, followed by the lysis of the host cell. This will release many viral copies and increase the impact of infection.

The upper respiratory tract hosts abundant microbiota which are symbiotic, pathogenic and commensal communities of microorganisms. This includes *Staphylococcus epidermidis*, *Corynebacterium* spp., and *Haemophilus* spp. While the lower respiratory tract is scarcely populated with microbes including species of *Pseudomonas*, *Streptococcus*, and *Veillonella*.These microbes are opportunistic pathogens and cause infection only under certain conditions. In order to cause infection, they overcome the host's immune system and producing adhesins which facilitates their adherence to the epithelial cells of respiratory tract. Some of them also produces polysaccharide capsules which allows them to evade phagocytosis by macrophages of the immune system. They produce endotoxins (produced by gram-negative bacteria) and exotoxins which results in the inflammatory responses in the host. They multiply rapidly and cause further infection. They also disable the mucociliary escalator. This results in the failure of the elimination of pathogens entering the respiratory tract.

DEFENCE MECHANISM AGAINST RESPIRATORY PATHOGENS

Upper respiratory tract which includes trachea, nasopharynx and bronchioles is the major site for respiratory pathogens to cause infection and replicate. Whereas alveoli in lower respiratory tract have relatively lower pathogens. Hence these are the primary sites where effective immune responses takes place, to kill the pathogens and stop the infection to spread further. For this, it mainly depends on innate immune system, which consists of:

i. **Pulmonary Epithelium:** It act as a physical barrier between the lumen (of the conducting airways and alveoli) and the vasculature. It not only prevents the colonization and spreading of pathogens in lungs but also prevents the fluid built-up inside the lungs. It forms a barrier by forming tight junctions which involves claudins, occludins and adherens. However, some pathogens can impair these proteins, resulting in the disintegration of the tight junctions.

ii. **Cilia and mucous:** These are minute muscular hair-like projections present on the epithelial cells that line the conducting airways including nostrils, trachea, bronchi and bronchioles. They secrete mucous which covers the respiratory tract and traps the pathogens entering the airways. The cilia also move the mucous and the pathogens trapped in it in upward direction. This phenomenon is known as mucociliary escalator. It eliminates the pathogens out of the body by coughing. The mucous also prevents the bacterial cells to adhere to the epithelial cells.

iii. **Pattern recognition receptors (PRRs):** Sometimes, the loss of epithelium integrity results in spread of pathogens into the vasculature and fluid built-up resulting in edema in lungs. Hence it is required to identify any signs of infection on the apical surface of the pulmonary epithelium. This is done by PRRs. Pulmonary epithelium along with some innate immune cells, expresses some toll-like receptors (TLR1-TLR10) which are specific to different antigens. They recognise different pathogens and produce cytokine and chemokine response.

iv. **Alveolar macrophages:** As mucous and cilia are not present in the alveoli which is the main part for exchange of gases, hence, some macrophages along with some lymphocytes and neutrophils are present there. Particles that are 1µm in size (or less) enter the alveoli of the lungs and interact with alveolar macrophages and fluids. These particles are then identified by these leukocytes. These macrophages ingest any foreign particle present inside the lungs by binding to those pathogens and phagocytizing them.

v. **Alveolar fluids:** Alveolar fluids and some constituent fluids present in the conducting airways contains some lysozymes, which is responsible for the lysis of bacterial cells and lactoferrin, which removes iron from the bacterial metabolism. Apart from it, this fluid also contains immunoglobins like IgA and IgG, and some antibacterial peptides called defensins, which are released from the leukocytes and epithelial cells of the respiratory tract. The defensins are involved in the host defence mechanism and immune signalling activities. Some surfactant- associated proteins are also present in this fluid that act as microbial opsonins. These opsonins are responsible for promoting the activity of the macrophages and enhance the phagocytosis of the bacterial cells.

vi. **T cells:** T helper cells produces cytokines in response to pathogens present in the lungs and promote the proliferation of epithelial cells.

THERAPEUTICS USED AGAINST RESPIRATORY PATHOGENS

Therapeutics are used to treat diseases and its signs and symptoms in patients. Although, vaccination is the most effective way to treat these diseases, but there are still many challenges regarding this issue because some viruses like influenza virus that undergoes mutations at a very rapid pace, shows resistance to such anti-viral therapy. Hence, this requires the need to re-formulate vaccines at least once in a year.

With advancement in research and drug development, certain biological molecules are discovered that have the potential to block the replication of virus in the host. These molecules are known as therapeutic agents and involves macromolecules, microbes, fungi and many more. There are various therapeutic strategies that are currently being used against respiratory viruses that are responsible for causing diseases like influenza, RSV, SARS-CoV, MERS-CoV. Some of them are:

1. Nucleic Acid Based Therapeutics
2. Microbial Therapeutics
3. Antiviral Agents
4. IgY-based Therapy
5. Peptide-based therapeutics

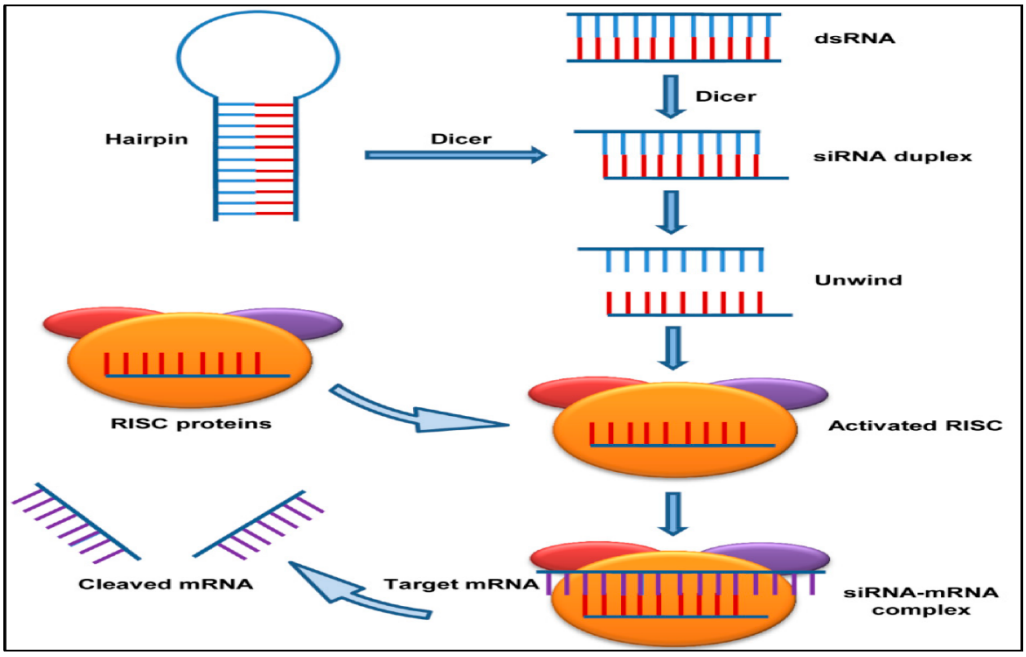
NUCLEIC ACID BASED THERAPEUTICS

These therapeutics involves the use of nucleic acids and are classified into DNA- based therapeutics and RNA- based therapeutics. These molecules have the potential to supress the virulent genes by either RNA interference or catalytic cleavage of the transcripts of the pathogen.

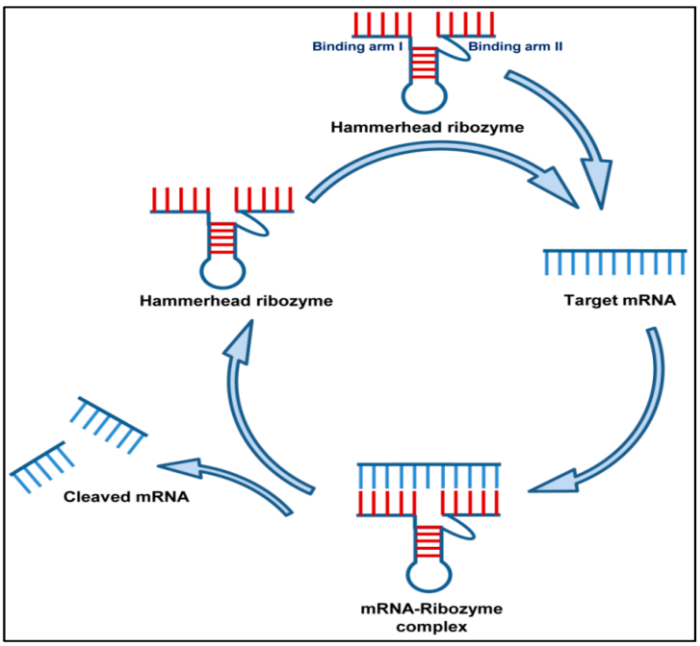
RNA-based Therapeutics: Scientists have discovered small RNAs that are able to control the expression of some specific genes present in the eukaryotic genome. Such RNAs are called short interfering RNAs (siRNAs) and microRNAs (miRNAs). They can deactivate the mRNAs of some specific virulent genes by forming complementary nucleotide sequences. This prevents the translation of those genes hereby, preventing their expression in host.

The siRNAs are small double-stranded RNA molecules containing 20-30 nucleotides that play a major role in post-transcriptional gene silencing in eukaryotes. It has also been discovered that synthetic siRNAs are capable of inducing RNA interference in mammals. These segments interrupt the expression of certain target genes by forming complementary nucleotide sequences. Thus, degrading their mRNA. These RNAi molecules can be efficiently designed to target only specific genes of respiratory viral mRNA. Therefore, they can also be used against mutated viruses instead of many other antiviral drugs. The dsRNA is introduced into the cell with the help of a vector or transposons. It is identified by RNA binding domain (RBD), having an endonuclease catalytic domain. This dsRNA undergoes cleavage to form siRNA molecules which bounds to the RNA induced silencing complex (RISC), a part of enzyme dicer. One of the strands of siRNA then forces RISC on target mRNA. After this, the dicer releases some protein that slice the mRNA, resulting in its suppression and preventing the protein synthesis.

Ribozymes are RNA molecules that have the catalytic activity and are quite like DNAzymes. Ribozymes (Rz) are naturally occurring molecules that play a major role in the cleavage of phosphodiester bonds of RNA. Out of all the classes of ribozymes, only hairpin and hammerhead ribozymes are used for cleaving the target RNA. They effectively reduce and prevents replication in viruses having RNA genome which results in the inhibition of pre-genomic RNA levels of pathogenic viruses. To overcome the challenges occurring due to mutations in RNA viruses, ribozymes present in the host cells are used efficiently as therapeutic agents. Various in-vitro and in-vivo experiments are being performed by the scientists which shows the effectiveness of ribozymes. One of the experiments showed better results with hammerhead Rz instead of hairpin Rz in the inhibition of influenza A virus by cleaving the viral RNA-segment. Another study involved the development of SOFA-HDV-Ribozymes and showed its effect on recognition of specific viral sequences in influenza virus and cleavage its viral mRNA. This eventually resulted in inhibition of viral replication. Another study performed on SARS-coronavirus involves the use of a chimeric DNA–RNA hammerhead ribozyme. This Rz was successful in inhibiting the viral replication upto 60%.



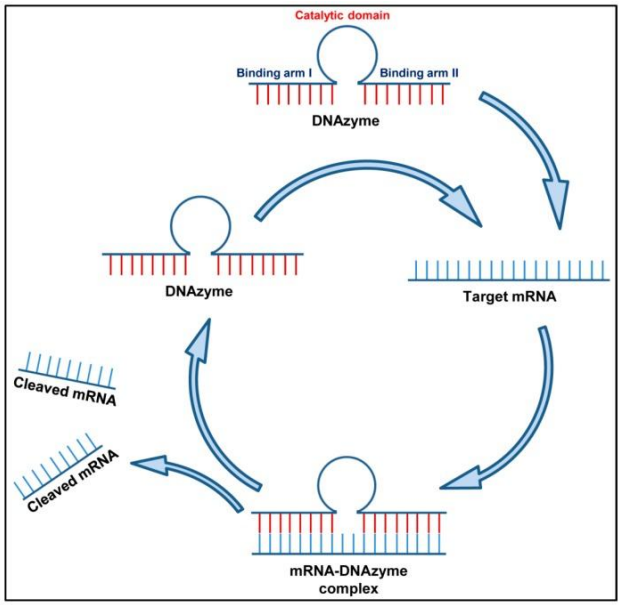
Mechanism of Hairpin ribozymes



Mechanism of hammerhead ribozymes

DNA-based Therapeutics: With advancement in science and technology, DNA is now recognized as a therapeutic agent. Deoxyribozymes (Dz) or DNA enzymes, are single stranded DNA molecules that are synthesised and can recognize certain sequences in mRNA and cleaving them. A Dz molecule has a central catalytic domain having two arms (I and II). These arms are designed to have complementary sequences to the target mRNA and hence binds with it. They have an advantage over drugs as they provide high specificity and only targets the specific sequence of the target mRNA. A prototype of DNAzymes, which is also denoted as "10–23", was used to target the PB2 mRNA of the influenza A virus, hence inhibiting its replication within patients.

A study showed that both ribozymes and DNAzymes are used simultaneously to inhibit the replication of some respiratory viruses like influenza virus upto 54%. Some researchers also concluded that combining DNAzymes and Ribozymes can result in the development of an effective therapeutic agent which can result in the better gene suppression and inhibiting the viral replication in host cells. DNAzymes are also synthesised in-vitro to silence RSV N, M2 and F genes of Respiratory Syncytial Virus (RSV). They were successful in cleaving the RNA of the virus and inhibiting its gene expression of F viral gene. Certain experiments are also conducted to target SARS-CoV by DNAzymes molecules. A DNAzyme is used to target the 5'-UTR (untranslated region) of the SARS-CoV genome to inhibit its replication inside host cells. The DNAzyme-104 showed successful cleavage of SARS-CoV RNA, thereby reducing its expression in mammalian cells. This study showed the basis of use of DNAzymes against SARS-CoV.



Mechanism of DNAzymes

Antisense Oligonucleotides (ASOs) are another example of DNA- based therapeutics. They are synthetic single stranded DNA molecules containing 15-30 nucleotides. They bind to the complementary sequences of the target RNA and inhibits its function in the host cells by either cleaving it or inhibiting the translation of mRNA. They are highly specific in inhibiting the gene expression in viruses. In a study, some antisense oligonucleotides were designed by researchers against influenza A virus genome to inhibit its replication in host cells. ASOs are also used to target Respiratory Syncytial Virus to inhibit its replication in-vitro. For this, Human epithelial type 2 cells were infected by RSV strain A2 and targeted with the desired ASOs. The targeted RNA was successfully cleaved by the ASOs at specific antisense oligonucleotide binding site and resulted in the inhibition of the viral antigen in the infected cells. This showed that ASOs can be used as a therapeutic agent against respiratory pathogens including SARS-CoA, RSV, Influenza A virus and many more.

MICROBIAL THERAPEUTICS

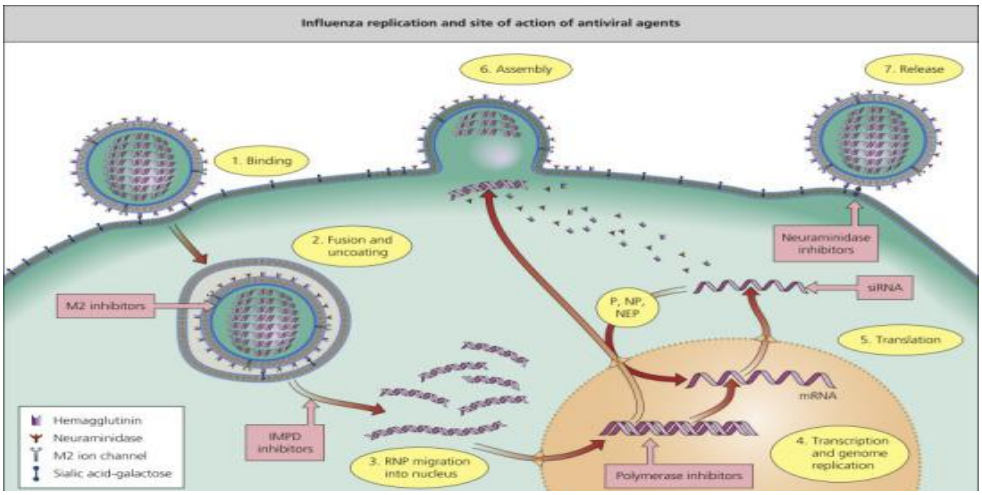
Many studies and research suggest that microbial therapeutics can prevent and treat respiratory diseases caused by viruses. They show better results when applied directly to the conducting airways of the respiratory system. The bacteria administered in the airways of respiratory system undergoes multifactorial bacterial mechanisms and reduces the viral replication and the severity caused by them. Sometimes viral infections can cause immune dysfunction and can lead to upper and lower respiratory tract (URT and LRT) superinfection by the microbiota that are already present there. The microbiota contains several opportunistic bacterial pathogens like *Staphylococcus aureus*, *H. influenzae* and *Streptococcus pneumoniae*. The bacterial microbiota can highly impact the viral infection as they interact with the virus as well as with the host on the mucosal surface of the respiratory tract and can determine the course and condition of the infection caused by the virus.

After the entry of virus through the mucous layer of the respiratory tract, the innate immune system gets activated. Pattern-recognition receptors (PRRs), present on host cells, identifies the microbe-associated molecular patterns (MAMPs) that are released by the infected cells. Some MAMPs are shared by both bacteria as well as viruses and some of these are also used as immunomodulatory agents in the gut. Hence, some beneficial bacteria are used to stimulate PRRs and induce signalling that are involved in anti-viral actions in the host. Therefore, such bacteria prevent the immune dysfunction occurring in SARS-CoV-2 infection, with the help of MAMP-PRR interaction in the host. This results in the production of probiotic factors.

Many studies involve the oral delivery of bacterial probiotics (live microbes that are beneficial to host's health) as a part of microbial therapeutics. This involves effects in the airways by signalling between microbiota in the airways and immune stimulation in the gut and airway immunity. Apart from this, probiotics are also administered locally in the airways and is more efficient way to modulate the airway microbiota. Microbial therapeutics that are administered locally can inhibit the pathogens and reducing the inflammation in the airway by gut–respiratory axis which is caused by targeted mucosal immune responses in the respiratory airways. These locally administered probiotics act as prophylaxis against the respiratory infections caused by viruses and other subsequent infections.

One of the approaches involve the inhibition of virus binding to the host cells. It is assumed that probiotics can block the binding of viruses to the host cells by some blocking mechanisms. The mechanism involves direct interaction of microbes with viral particles and trapping them or inhibiting their adhesion to the host cells by binding themselves to the viral receptors. Some studies show that *Staphylococcus epidermidis* ATCC12228 can bind with influenza H1N1 viral particles, showing effective anti-viral activities. Some experiments also showed that bacterial lipopolysaccharides (LPS) reduce the stability of influenza strains in human as well as birds. Whereas other experiments showed increased stability of virions, their infectivity and co-infection of enteric viruses after binding to bacterial LPS. However, these differences can be observed due to differences in bacterial LPS or their cell wall composition.

Apart from blocking the binding sites of virus, these bacteria can also disable the viral proteins that are responsible for causing infection. Lactic acid bacteria (LAB) have some antiviral properties because it secretes D- and L- lactic acid which is responsible for reducing the virulency of influenza A virus. Some bacteria like LAB and *Enterococcus faecium* secretes bacteriocins (antimicrobial peptides that are synthesised by ribosomes) and shows antiviral properties. Some experiments shows that *Streptomyces* along with *Rothia*,of the respiratory system, produces valinomycin which is effective against RSV, MERS-CoV and SARS-CoV**.**



Another approach of microbial therapeutics involves the genetic modification of the bacteria in probiotics. For example, Griffithsin, a protein extracted from Griffithsia (red algae), helps in inhibiting SARS-CoV in mouse models during experiments. Hence, developing Griffithsin-producing bacterial strain can be effective against coronaviruses. Studies show that genetically modified microbial therapeutics are efficient against respiratory viral infections especially in upper respiratory tract. Recombinant microbial therapeutics that are administered intranasally are better than the conventional methods because it has less side-effects, provides long-term immunity and require a lower dose. It was observed that intranasal*Limosilactobacillus fermentum*CJL-112L enhanced the production of IgA against the influenza virus in mice model.

IgY-BASED THERAPY

Even though, using antibiotics and antiviral agents against respiratory pathogens are effective but to a certain extent only because of the growing resistance in pathogens against these agents. Vaccination also faces many challenges including the antigenic variations in different viral strains. Hence, passive immunization is an effective and alternative way to treat respiratory infections in immune-suppressive individuals. For this, polyclonal or monoclonal antibodies (extracted from convalescent sera, or from the sera of immunized animal and humans) are used. Egg IgY antibodies are used against bacterial as well as viral infections. IgYs are mainly produced by birds, reptiles, and amphibians, and are quite similar in the function as IgGs in mammals. Using IgY antibodies have many benefits including the fact that it is well tolerated in humans. It can also be used in patients having allergy against egg albumin because IgYs are purified and are devoid of egg albumin. IgYs does not interact with human complement system or Fc receptors and does not cause any inflammation.

IgYs bind to the bacteria and viruses, eliminates them through gut and prevents their replication inside the host. IgYs causes agglutination of pathogens, which resulting in their immobilization and removal from the body. It is also responsible for blocking the adhesion of the pathogens to the host cells. It has been found that IgYs bind to the growth-related components like fimbriae, lipopolysaccharides, and outer membrane proteins of bacterial pathogens. This results in their inhibition, alteration in cell signalling processes and lower production of toxin and its release into the host. Many studies also show that IgYs are responsible for the enhanced activity of phagocytic cells against pathogens. For example, IgY showed enhanced phagocytosis against *Staphylococcus aureus* by neutrophils. Other studies showed that IgYs are responsible for binding on the surface of pathogens like *Salmonella typhimurium* and causing some structural changes in bacteria which increases its chances of phagocytosis. IgY can neutralize the bacterial toxins and inhibiting the viral colonization by preventing the intercellular viral spread.

IgY antibodies are administered in the non-immune subjects by systemic, intravenous, or oral routes. These antibodies provide protection for a very short period (2 weeks). Hence, continuous supply of antibodies is required after every 2 weeks. This is only possible when there is large production of antibodies, which is done via hyperimmunization of chickens. For this, hens are frequently exposed to specific antigens, so that there is continuous production of IgY antibodies in the egg yolk, from where the antibodies can be extracted and purified. Antibodies that are transferred by intravenous route, shows better results in humans against respiratory infections, including influenza A and B viruses, RSV, Coxsackie virus, and rhinoviruses.

Using IgY antibodies have many advantages in the field of immunotherapy. They have rapid action than IgG antibodies and can be used in infants as well as immune-suppressive individuals. Studies suggest that IgY-based therapy is more effective due to the presence of sialic acid in larger quantity. The sialic acid helps in increasing the half-life of the drugs and hence, their efficacy. They are non-toxic, cheaper and can be produce at a faster rate. They also require exposure to lower quantity of antigens to produce and effective immune response.

PEPTIDE-BASED THERAPEUTICS

This is based on the peptidic macrocyclic compounds that can inhibit the fusion of respiratory viruses like influenza A virus. These peptides are similar in function to broad neutralizing antibodies (bnAbs). They bind to the Hemagglutinin (HA) stem and reduce the production of escape mutants. HA is a trimeric protein consisting of subunits that have HA1 and HA2 subdomains. HA1 is responsible for adherence of virus to sialylated receptors present at the surface of the host cell and HA2 is responsible for the fusion of the viral membrane with the host cell membrane. HA2 undergoes certain structural changes due to the low pH of endosomes. This structural change in the HA results in the fusion of the cell membrane of virus and host cell. Scientists thought of an innovative idea which involved the binding of a specific peptide to the HA stem region prior to the fusion of membranes.

Finally, by combining various strategies, a peptide called P7 was designed. It binds to the HA stem of several subtypes of influenza A virus including H1NI and H5N1 and inhibits the fusion of viral and host cell membrane. Studies showed that the peptide is highly stable and is non-toxic inside humans. Despite of being smaller in size than bnAbs, this peptide covers the whole region of HA stem which is used to inhibit the viral fusion to the host cell.

Another peptide which acts as an inhibitor is P9 which is also effective against IAV. This peptide is extracted from mouse β-defensin-4 (mBD4). The peptide has two sulphide bonds along with many basic amino acids. It binds to the glycoproteins of the virus and prevents RNA replication. This is possible by preventing the pH drop due to presence of high amount of basic amino acids. Hence, it also prevents the fusion of viral membrane with the host cell. Some peptides that are extracted from heptad repeat 2 (HR2), target the transition period between prefusion and post fusion of RSV in host cells. Two peptides (T108 and T118) are identified from the HR2 sequence and can inhibit the formation of virus-mediated syncytia. Apart from this, peptides that are obtained from the HR2 domain, can be used as inhibitors of other respiratory pathogens. For example, a peptide having 36 amino acids is obtained from HR2 domain of human parainfluenza 3 (HIPV3), can successfully inhibit HIPV3 as well as Hendraand Nipah virus.

In some cases, these peptides can be easily degraded by proteases and cannot be administered orally. They are expensive and some experiments shows that they do not cross the epithelial as well as skin barriers. Hence, many research and development are being carried out in the field of peptide therapeutics to overcome their limitations.

VACCINES AGAINST RESPIRATORY PATHOGENS

Majority of respiratory infections are caused by viruses, but the focus of vaccines is to target the most common viral pathogens. We only have influenza virus vaccine and adenovirus type 4 and 7 vaccine. However, many studies are being conducted to develop vaccines against other viruses like RSV, parainfluenza virus and many more. This arises a major concern as there are some obstacles that needed to be overcome in the field of vaccine development. One of the challenges is that vaccines are highly needed for the high-risk groups, that are infants and old people. Other challenges include inducing immunity by providing more than one dose of vaccine and reducing the risk of reinfection after primary infection by the pathogens. Vaccination is the most effective and affordable way to prevent and provide protection against respiratory viruses. Some improved and effective vaccine approaches that are used against respiratory pathogens are:

- Nanoparticles-based vaccines
- Intranasal DNA vaccines
- Recombinant BCG vaccine

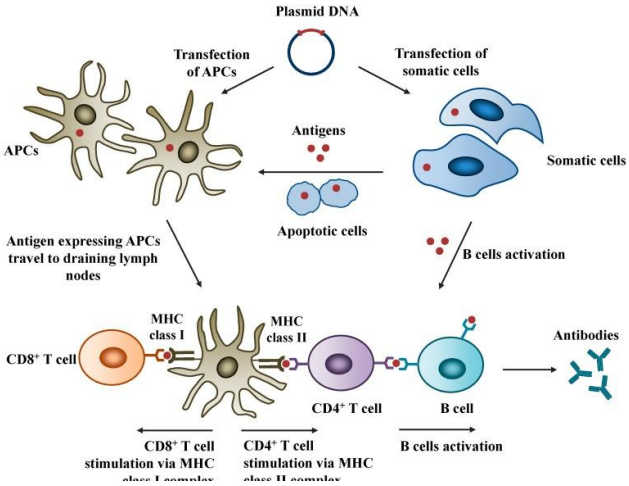
NANOPARTICLE-BASED VACCINES

It is observed that conventional vaccines that involve live-attenuated pathogens may cause reversion of virulency in pathogens while the ones that involve killed and inactivated pathogens provide low immunity. To overcome this issue, nanoparticles-based vaccines are developed. We can use desired antigens for developing nano vaccines instead of conventional vaccines. These nanoparticles can be synthesised or extracted from organisms and includes liposomes and certain virus-like particles to carry antigenic molecules. They can be administered via sub-cutaneous, intramuscular, oral, or intranasalinjections. Some developments have been made to improve the physiochemical properties of nanoparticles like size, shape, solubility, surface chemistry, and hydrophilicity.

Antigens can be incorporated in desired nanoparticles by encapsulation or by conjugation. The main advantage of using nanoparticles as vaccines is their exceptionally small size and the ease of their entry in viruses which are also nano sized. They also provide protection to the antigens from proteolytic degradation inside the body and ensures long lasting presenting of antigens to antigen-presenting cells (APCs). Some of the commonly used nanoparticle that are used for the development of vaccines are carbon nanotubes (CNTs), carbon black nanoparticles, poly (lactic-co-glycolic acid) (PLGA), polystyrene nanoparticles, titanium dioxide (TiO_2) nanoparticles, silicon dioxide (SiO_2) nanoparticles, and aluminium oxyhydroxide nanoparticles. After the nanoparticles are taken up by APCs, they destabilizelysosomes which results in the release of its contents like cysteine protease cathepsin B. This is identified by the NLRP3, which leads to the formation of inflammasome complex. This activates immune cells because of production of interleukins. This shows that nanoparticles when used as vaccines can activate immune cells and hence, are immunogenic in nature.

Nasopharyngeal-associated lymphoid tissue (NALT) is the primary site where antigens enter the host body and is major site where immune response takes place. Hence, nano-vaccines are designed in such a way that they mimic the respiratory viruses and follow their pathway to produce effective immune responses. For this, scientists make sure that nanoparticles should be 20–200 nm in diameter (like that of respiratory viruses) and positively charged, because they have been reported to produce better immune responses than negatively charged ones. They are administered orally or intranasally because they provide strong and effective protection as compared to when they are administered viaSubcutaneous or intramuscular injections. Apart from so many advantages, they have some limitations also which includes less antigen loading, low synthesis yield, poor targeting capability to immune cells and limited manufacturability.

INTRANASAL DNA VACCINE



Mechanism of Intranasal DNA Vaccines against respiratory pathogens

DNA vaccines that are administered nasally, have many advantages as it can trigger the immune responses better than the vaccines that are administered via any other route. These vaccines also provide protection against respiratory infections like coronavirus, RSV, influenza and many more. This strategy depends on the production of target antigensin-situ.

This includes the delivery of plasmid DNA encoded antigenic proteins to specific tissues. The plasmid DNA expresses the antigen in the individual and triggers immune responses that provide protection. This technique has been used for encoding various bacterial and viral antigens and are then tested for their efficiency. These vaccines are cheap, easily manufactured and highly stable at high temperatures. But they are not able to provide enough protection against pathogens. It is observed that these vaccines are not able to produce T cell response good enough to provide protection, on their own. The evaluation of one such plasmid vaccine pTHr DNA HIV-1, showed very weak immunogenicity in healthy humans.

After administration, many somatic cells and APCs get infected. The antigens stimulate both cellular and humoral immune responses. Antigen expressed APCs migrate to the lymph nodes and interact with antigenic peptide-MHC and stimulate T cells. Simultaneously, B cells are also activated and antibodies are produced. They also activateCD8+ T-cells, that helps in controlling infections

RECOMBINANT BCG VACCINE

Bacille Calmette Guérin (BCG) vaccine is quite immunogenic and stable. But many approaches have been made to develop BCG as a recombinant vector, so that it can express foreign genes and produce effective immune responses against diseases like RSV and metapneumovirus.

It is resistant to antibiotics and are not easily transformed into a recombinant DNA.After many research and development, some rBCG stains aredeveloped that are used to induce immunity in animal models. These recombinant vaccines can express nucleoprotein and the phosphoprotein of RSV and hMPV, respectively, and induce long lasting humoral as well as cellular immunity against these antigens in mice. They also induce $CD4^+$ and $CD8^+$ T cell responses in the body. $CD4^+$ are T helper cells and kill the antigens, while $CD8^+$are T killer cells that kills the cancerous cells. The rBCG technology is also used to treat other infections like pertussis, hMPV and many more.

One recent approach has been made against SARS-CoV-2 using rBCG. This will enhance innate as well as adaptive immune responses when the antigen is expressed in the body. The hypothesis involves the delivery of antigens to the lymphoid tissues and inducing systemic and pulmonary immunity by activating T cells. Following this strategy, a rBCG vaccine called VPM1002, has been designed and is subjected to clinical trials, for reducing SARS-CoV-2. It was observed in some preclinical studies thatVPM1002 is more immunogenic and safer.

CONCLUSION

With increasing rate of respiratory infections and growing mortality as well as morbidity rate, is a major concern for scientists around the world. This increases the demand of new technology by which we can design and develop innovative therapeutics and vaccines against respiratory pathogens. This also means that these available therapeutics and vaccines needed to be reformulated because many pathogens become resistant to them.

But we still do not have improved much in this field and that is why there are no vaccines available for some common diseases like RSV and common cold. Although several studies are being done to develop new vaccines and improve the existing ones. Some viruses like RSV and influenza change their pattern after every year which makes it quite difficult for scientists to examine their genome and formulate a vaccine. Apart from vaccines, many therapeutics like nucleic acid-based therapeutics and IgY-based therapy are successful ones and safer than the conventional therapeutics. They are also non-toxic to humans because it is a main factor which contributes to an efficient therapeutic.

There is a lot more to be done in the field of vaccine technology and its development, to improve the quality of vaccines and therapeutics against respiratory pathogens. Some vaccines like nano vaccine are more efficient, non-toxic, and safer and involves innovative use of nanotechnology and vaccine technology. This is necessary for the development of vaccines in future.

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