Importance of Genetic Engineering and Gene Therapy in the Management of Viral Diseases and Outbreaks

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ABSTRACT

BACKGROUND

Emerging globalisation over the years has brought a lot of opportunities in every corner of the world. The economy is now evolving, and things are getting easier for the public. But with all these affirmations, the spread of diseases all around the world has become easier and harder to contain. Epidemics usually result in pandemics due to the constant movement of the people from one part of the world to other. With the recent rapid onset of the COVID-19 pandemic, the world has come to realise the inadvertent and unequal nature of the severity, and spread in the region of the disease. Hence, it is fair to say that such outbreaks carry the potential to cause a catastrophe on the global economy and livelihood. A changing environment and landscape, as we know, can cater to the fitness and development of new and accomplished viral diseases, and in today's era, with factors such as increasing population, tropical deforestation and rapid industrialisation, the viruses tend to adapt and exploit the replication of their genomes. Hence, leading to severe conditions in the human body. Some of the notable viral outbreaks include severe acute respiratory syndrome (SARS), HIV-AIDS, Ebola and the ongoing COVID-19. Genetic therapy is an extensively specific and elaborate stream which deals with manipulation of the human genes and is being considered as a form of a promising alternate medicine. The management of infectious diseases demands the introduction of specifically designed genes to either inhibit or block the gene expression to manoeuvre the functioning of the infectious agents. The following review discusses some of the most recent and notable outbreaks, their symptoms and the how advancements in genetic therapy came into play in treating, nullifying and managing them along with the rationale of the prospects and problems faced by the scientists over the years in developing vaccinations, treatment modalities and new controlled procedures in regulations of such outbreaks all over the world.

KEY WORDS

Pandemic, Genetic Engineering, Molecular Therapy, Immunotherapy, Reverse Genetics, RNA Decoys, HIV-AIDS, Ebola Virus, SARS, H1N1 Influenza, COVID-19

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BACKGROUND

21st century has seen a lot of development in terms of economy, healthcare, trade and other services. The world has become a smaller place to live in with increasing convenience of international boundaries getting blurry and globalisation taking the throne. Although, as every coin has two sides, one of the biggest culprits of converting new diseases into pandemics is also, unfortunately globalisation.¹ Infectious diseases especially of the viral origin spread like wildfire and is considered the most common reason for disruption of livelihood, trades and the overall economy.² Diseases like Ebola, Lassa, Yellow Fever, Zika, Chikungunya, the H1N1 influenza, Middle East Respiratory Syndrome (MERS), SARS, cholera and the ongoing COVID-19 are some of the viral outbreaks that have surfaced into the population in this millennium.³

In 1798, the inoculation of humans against the smallpox with cowpox, a less virulent substitute, viral infections were solely controlled by vaccinations and made a majority of first line treatment since, last two centuries.⁴ Even though diagnosis and first-hand treatment are usually symptomatic relief and vaccinations, a rapid growth in the biotechnological arena has introduced the role of genetic manipulation into the treatment of such outbreaks which has made a major impact in the treatment of the diseases.⁵ Genetic manipulation has been emerging as one of the main foundation of techniques in gene therapy and virology to counter diseases and control symptoms. This is done by inserting, deleting or replacing nucleotides for specified locations in the genetic map of the subject.⁶ Gene therapies have actively been used in treatment of viral diseases like influenza and HIV,⁷ and many viral interventions have helped in the treatment of other diseases like tuberculosis and malaria in recent times.⁸ Molecular genetic techniques have been growing and evolving based on the mechanism of action of viruses and the gene expressions they affect, along with the basic morphology of the cellular components of the micro-organism.⁹ The techniques have been combined in the given table.

GENE THERAPY IN THE TREATMENT OF VARIOUS EPIDEMICS AND PANDEMICS

Human Immuno Deficiency Virus

Acquired Human Immuno Deficiency Syndrome is caused by HIV 1 of the lentivirus family. Discovered in the 1980s, this disease shows very less or mild influenza like signs and symptoms at initial stages which can develop into severe nausea, vomiting and since the disease causes reduction of immune system, diseases like Kaposi sarcoma and tuberculosis are very commonly seen.¹³ Genetic intervention in the treatment of HIV-AIDS has proven to be a paradigm for the field.¹⁴ Belonging to the lentivirus family, this virus consists of the RNA genome which is reverse transcribed into DNA by reverse transcriptase protein. Theoretically, the viral genome has 3 sets of proteins, which if interrupted or inhibited can disrupt the life cycle of HIV. The treatment options include¹⁵

- a. Development of potent anti-HIV 1 transgenes - which can be injected at any stage to disrupt the replication of the HIV 1 virion which is infective in nature.

- b. Protein Strategies - include genetically engineered trans dominant negative mutants, intrakine toxins and single chain antibodies to inhibit HIV-1 virion. DNA vaccines are also being developed for the same.
- c. RNA Strategies - include use of antisense RNA, ribozymes, RNA decoys and interference RNA commonly called RNAi.

Ebola

Coming from the family of Filoviridae, Ebolavirus was discovered in 1977 as a severe disease affecting the humans. Many outbreaks have occurred in different parts of the world especially in the sub-Saharan region, most recent being the 2018 outbreak in Democratic Republic of Congo affecting about 29 health zones and is recorded as the largest outbreak since the virus's discovery.¹⁶ The disease spreads from direct contact, either with the infected or from the objects belonging to the infected individual. Symptoms of the disease progress from dry symptoms namely fever, cough and malaise to considerably wet symptoms like diarrhoea and vomiting that can become fatal if left untreated. With vaccinations being mandatory for the prevention of the spread of the disease, the treatment of the disease has taken an enhanced path in genetic engineering.¹⁷ Techniques produced to counter Ebola virus include

Reverse Genetics

This technique consists of development of the filovirus with the key gene sequence, but which isn't recombinant hence, isn't infectious in nature. Similar studies have been done and trials have performed using the Rabies Virus (RABV) which has expressed glycoproteins for both rabies and Ebola, hence, inducing humoral immunity against the two and confer protection of the patient.¹⁸

Nucleoside Analog Candidate Therapies

These are safe and effective antiviral agents that are designed and engineered to be used as post exposure treatment.

RNA-Silencing Molecules and Antisense Oligomers

Also commonly referred to as RNA interfering molecules which are targeted to disrupt the cell differentiation and development of the virus. These are very commonly termed as siRNA and renamed as TKM Ebola which function to target the polymerase (L) gene of ZEBOV which has successful clinical trials and is used efficiently for treatment.²⁰

Immunotherapeutics

The idea of using passive immunity for treatment of this disease goes as long as 1995 in Zaire, although many trials have been unsuccessful until recent times, where a novel immunotherapeutic, a combination of monoclonal antibodies named (Zmapp)²¹ has gained success in animal trials and is currently undergoing clinical trials in the States.

SARS (Severe Acute Respiratory Syndrome)

SARS-CoV also called as SARS coronavirus was first reported in 2002 in Southern China which later affected 26 countries, making a mark in the list of pandemics that hit the world in 21st century. It was an airborne disease which is spread person to person.

Nucleic Acid Based Genetic Therapy	Description	Advantages	Disadvantages
1. Antisense RNA and DNA	These help to block occurring gene expression by nucleic acid base pairing (Watson and Crick Model)	These are up taken easily by the host without any immunologic complication.	Stability of the oligonucleotides used esp. in antisense DNA still remains controversial.
2. Ribozymes	These are antisense RNA molecules which also have catalytic activity present in them, they help in inhibition by binding to the target cells to undergo a specific hybridization, cleaving the phosphor-di-ester bond present.	The major advantage is that these are not consumed during the reaction, hence one single ribozyme can help inhibit several molecules	The catalyst activity extent of the Ribozymes is still unknown.
3. RNA Decoys	These are overexpressed short strands of RNA molecules which are substitutes for cis-acting elements, acting as decoys for the trans activating proteins hence preventing the binding of both in viral genome.	These are rarely affected by any mutation in the targeted genome and hence be used in various stages of mutation of the genome.	The over expression of the RNA transcripts can have deleterious effects in cell functioning and harmony.

Table 1. Techniques Which Involve Nucleic Acid Components for Genetic Therapy.^{10,11}

Protein Based Approaches to Gene Therapy	Description	Advantages	Disadvantages
1. Trans-dominant Negative Proteins	These are mutant forms of different types of proteins processed in such a way that they help in inhibiting the infectious agent	Since these proteins compete for essential substrate with the target organelle, the mechanism of inhibition is comparatively easier.	These engineered cells have the tendency to pose immunogenicity i.e the immune system response can cause their own destruction
2. Anti-Infectious Cellular Proteins	These are basically derived from normal cellular components but show specific gene inhibition activity	Engineered cells show non-antigenic activity i.e do not get eliminated by recipient's immune system.	May have shown success in-vitro but clinical trials still remain unsuccessful in case of HIV treatment
3. Single chain Antibodies-	These are in the form of single chain variable fragments belonging to an antibody which has the ability to retain entire antigen specificity and binding site capability of the parent gene.	These bind to the site and prevent gene function and cannot be secreted out.	Not recorded
4. Suicide Genes-	These genes are used in order to selectively kill the infectious cells in the host.	-	-

Table 2. Nucleic Acid Based Approaches to Gene Therapy^{10,12}

Immunotherapy	Description	Advantages	Disadvantages
1. DNA Vaccine	Generating an immune response against the native components and proteins of the infectious disease with the help of transmission of plasmid DNA into the host cells	i. easy to produce ii. effective building humoral and cytotoxic immunity due to presence of native proteins iii. target cell specificity is not important.	i. chances of affecting other cell types than targeted. ii. anti-DNA antibodies that can be produced iii. random integration of vaccinated DNA in target cells
2. Agent-Specific Cytotoxic T Cells	Ex-vivo expansion of patients own antibodies as a method of treating the disease	-	-

Table 3. Immunotherapeutic Components in Gene Therapy¹⁰

Name	Mechanism of Action	Status
Favipiravir	Induce viral mutagenesis hence reducing post exposure symptom severity.	Approved for use
JK-05	An antiviral compound designed to target RNA polymerase inhibiting viral replication.	Approved for emergency use only
BCX440	An adenosine analog which inhibits the function of viral RNA polymerase	Not approved

Table 4. Nucleoside Analog Candidates Used in Therapeutic Advancements Taken Against Ebola.¹⁹

Symptoms include fever, malaise, myalgia, diarrhoea which mimic flu like symptoms mostly. Vaccinations have not been developed yet but some genetic therapy has taken place to provide treatment and prevention of the spread of the virus.²² Currently, many studies are undergoing which use SiRNA duplexes (RNA interference genome) as an efficient prophylactic agent due to its antiviral efficacy and a comparatively lower therapeutic effect, but counter measures have produced alongside to increase the therapeutic properties too.²³

H1N1 Swine Flu (Influenza A)

A disease caused by the A type virus of the Orthomyxoviridae family, H1N1 influenza broke out to become one of the documented pandemics in 2009, attacking around 5 - 10 % adults and almost 20 - 30 % children.²⁴ With flu like symptoms, this disease is transmitted via droplets from unprotected cough and sneezes and is usually seen spreading in crowded places. Although the infected individuals range from

asymptomatic to mild symptomatic, the fatal complications of this disease include pneumonitis, otitis media and bacterial pneumonia.²⁵ As the virus contains high affinity towards mutation, targeted or specific gene sequencing seems tedious and ineffective in the case. Currently, neuraminidase inhibitors (NAIs) like oseltamivir, zanamivir and peramivir are widely used to treat H1N1.²⁶

Genetic therapies are available in the form of manipulating HA and NA functionality. MHAA4549A, MEDI8852, VIS-410, JNJ63623872, Favipiravir and JJ3297 are therapies approved which work by manipulating the functionality of HA and NA, hence, countering to the mutations and increasing the efficacy of the treatment.²⁷

COVID-19

Originated in Wuhan, China in the year 2019, COVID-19 disease is caused by a new specimen of the coronavirus family named as SARS-Cov2. This virus has caused an international havoc with the ongoing outbreak and one of the largest pandemics recorded till date in this millennium covering almost 180 countries.²⁸ The symptoms include fever, malaise, and dry cough. Severe cases if left undiagnosed can advance into pneumonia which might turn fatal.²⁹ There is currently no treatment found, neither therapeutic, nor prophylactic, many combination drugs are coming to relieve symptoms but none of them have become the first line treatment of the disease. Genetic considerations taken for the treatment of the disease

work around the immune response of the body to the virus, where studies are being done on-

- a. Innate Immune Response – For catering an anti-viral response the innate immune cells have to follow the pathogenic associated molecular pattern (PAMPs) upon the invasion of virus which is recognised by the endosomal RNA receptors followed by the downstream signalling cascade hence, activating the first line defence mechanism of the body to fight the virus.³⁰
- b. Adaptive Immune Response (for vaccine development) - The Th1 immune response is playing a very important role in the control of SRAS-CoV virus. The CD8 + T cell response is one of the major reasons to not make the viral infection exceed up to a lung pathology. This information will be useful while developing passive immunisation for the disease.³¹

PROSPECTS AND PROBLEMS IN GENE THERAPY

The rationale of gene therapy in treatment of viral outbreaks is that most of the genes that are tailored to enhance the function of immune response of the body. These genomes, nucleotides or proteins made reduce efficacy of the viral antigen by either inhibiting or disrupting its life cycle to relieve the symptoms caused in the human body.^{32,33}

Mostly the genomes are used as a therapeutic measure but due to increasing outbreaks of the diseases, prophylactic and preventive measures have also been emerging which include such genetic advances. But with every good intention there are some side effects attached to it, especially when genes are involved. Gene therapy is a young field, so with every study involved there are more failures than successes involved. Studies are time consuming, technique sensitive and do not have any guarantee to provide positive results. But with the new techniques emerging, competition rising, gene therapy holds potential to become the treatment of choice in the new millennium.³⁴

CONCLUSIONS

Emerging viral pandemics around the world have given rise to the introduction of genetic manipulations in the field of virology. From adding, deleting and replacing the genes, treatment options have been advanced for diseases like HIV-AIDS, SARS, Swine Flu and even the ongoing pandemic of COVID-19. The field being new, it still holds a lot of unexplored arenas which might prove to become fruitful for the mankind and change the paradigm of treatment.

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