WEEK FIVE: Therapeutic Development with Viruses

Overview of Viruses

Viruses ('rogue DNA') are parasites of the living that cannot make anything on their own, but instead use cell's materials to build themselves utilizing host metabolic enzymes and host ribosomes for protein synthesis. Structurally…

- Nucleic acid core of DNA or RNA
- Crucial virus-specific enzymes
- Capsid and sometimes outer lipid envelope
- Complete viral particle called a virion

Life cycle of HIV

- Attachment
- Fusion
- Reverse transcription
- Integration into the host genome
- Transcription
- Translation
- Virion assembly and budding
- Maturation

Viral replication

- Adsorption to and penetration into susceptible host cells
- Uncoating of viral nucleic acid
- Synthesis of early regulatory proteins e.g. nucleic acid polymerase
- Synthesis of RNA or DNA
- Synthesis of late, structural proteins
- Assembly (maturation) of viral particles
- Release from cell

Transmission

- Respiratory (Influenza A)
- Fecal-oral (Enterovirus)
- Blood-borne (Hep B)
- Sexual (HIV)
- Vectors (Rabies)

The Immune System

1st line of defense in the immune system is the skin. If a pathogen evades this defense it will come across the 2nd line of defense…

White blood cells include T-cells (natural killer cells) that recognize infected human cells and cancer cells. They will attack such cells and quickly kill them before searching for more cells to kill. The efforts of phagocytes (eat) and T-cells (kill) are known as the cell-mediated immune

system. The other part of the immune system is antibody-mediated immunity representing the 3rd line of defense (although most infections never make it past the 1st and 2nd defenses). If a pathogen makes it to the 3rd line of defense it triggers the production and release of antibodies, which are proteins that latch onto, damage, clump, and slow foreign particles. New particles take longer to identify and hence an individual will remain ill for longer, however old particles are quickly recognized (immunological memory).

Immunity can be active or passive. Active immunity is when you produce the antibodies, which is because your body has been exposed to the antigen in the past through exposure to the actual disease or planned exposure to a form of the antigen that has been killed or weakened. In this situation you have detected the antigen, eliminated it and remembered it. With planned exposure, vaccination, the pathogen has been killed or weakened to that minimal symptoms occur. This method of generating active immunity has eradicated or severely limited several diseases – e.g. Polio, Smallpox. Sometimes active immunity (like chicken pox) lasts a lifetime, but not always. This is why vaccinations often involve booster shots (to remind the immune system of that antigen).

In 1918 the Spanish Influenza spread across the globe and infected 20% of the human population, killing about 5% - 100 million people. These days transport and travel is far more accessible allowing a pathogen such as Influenza to spread and infect more people than in 1918.

In passive immunity it is not you that produces the antibodies. Instead, a mother passes immunities on to her baby during pregnancy. These antibodies will protect the baby for a short period of time following birth and passive immunity will last until the death of the antibodies. It could be a good area for therapeutic development as many pregnant mothers would be willing to get vaccinated as to pass on antibodies to their babies to protect their health.

Poliovirus

- Enterovirus
- RNA genome
- Faecal-oral transmission
- Gastrointestinal illness and poliomyelitis (affects CNS causing temporary or permanent paralysis)

Influenza A

- Myxovirus
- Enveloped virus with segmented RNA genome
- Infects wide range of animals other than humans
- Antigenic variation
- Respiratory transmission and infection
- Destruction of respiratory epithelium
- Secondary bacterial infections
- Cytokine release causing fever (IL-1 and IFN)
- Vaccination: developed yearly for predicted strain, epidemic occurs when prediction is wrong

Viruses Linked to Human Tumours

- Epstein-Barr Virus: Burkitt's lymphoma
- Human papillomavirus: benign warts and cervical carcinoma

- Human T-cell Leukaemia Virus (HTLV-1): leukaemia
- Hepatitis C Virus: Liver carcinoma

These tumours occur when the virus nucleic acid, as DNA, integrates into the cellular genome causing changes in gene expression that lead to uncontrolled cell multiplication and tumour formation.

Antiviral Therapy

To poison the virus is also to poison the host
…because viruses utilise so many host functions

Targets: attachment/entry, nucleic acid replication, virus protein processing, virus maturation

Viruses as Therapeutics

In the 1980s scientists began to look at gene therapy. They inserted human genes into bacterial cells and found that the cell would transcribe and translate the information into a protein, which could be introduced back into human cells.

Four Approaches to Gene Therapy

1. A normal gene is inserted to compensate for a nonfunctional gene
2. An abnormal gene is traded for a normal gene
3. An abnormal gene is repaired through selective reverse mutation
4. The regulation of gene pairs is changed

Vectors deliver the therapeutic gene into a patient's target cell, which becomes infected with that vector. The vectors genetic material is then inserted into the target cell, and function proteins are created from that gene causing the cell to return to a normal state.

The first case of gene therapy was performed on Ashanti DeSilva as a treatment for severe combined immunodeficiency in 1990. Doctors removed her white blood cells, inserted the missing gene into the WBCs, then put them back into her blood stream to strengthen her immune system. The problem was that it could only work for a few months.

Jesse Gelsinger, a patient lacking ornithine transcarbamylase, died in 1999 due to an unsuccessful gene therapy. Within hours after the doctors shot the normal ornithine transcarbamylase gene in a vector into his liver, he developed a high fever and his immune system began racing our of control. His blood began to clot, ammonia levels climbed, liver hemorrhaged and a flood of WBCs shut down his lungs.

There have been successes in gene therapy with infants suffering from severe combined immunodeficiency, which is a profound deficiency in lymphocytes. As a result sufferers are unable to mount an adaptive immune response, thus are susceptible to infections. It is inherited as an X-linked disease (for practical purposes only affects boys) or as autosomal recessive. There are several abnormalities in the immune system when the defective gene is encoded on an autosome. To treat for immunodeficiency caused by a deficiency in adenosine deaminase in children, gene therapy was used with success. Mature peripheral T cells were transduced with a vector with the corrective gene, because mature peripheral cells were used as opposed to stem cells the procedure was labor intensive and had to be repeated many times.

Types of Viruses for Vectors

Retroviruses

- Create ds-DNA from RNA genome through reverse transcription using reverse transcriptase
- ds-DNA integrates into the human genome using integrase
  - Integrase inserts the gene anywhere because it has no specific site
  - May cause insertional mutagenesis: one gene disrupts another genes code, which can be severe if it disrupts cell division causing uncontrolled cell division and cancer
- HIV being evaluated for safety

Adenoviruses

- ds-DNA genome
- Respiratory, intestinal and eye infections
- Inserted DNA not incorporated into genome and not replicated hence would have to be reinserted when more cells divid
- Common cold

Adeno-associated Virus

- Small ss-DNA that inserts genetic material at specific point on a chromosome
- Parvovirus family – causes no known disease and doesn't trigger immune response
- Low information capacity
- Gene always 'on' – not always ideal
- Useful for hemophilia treatments, injected into muscle prompting cells to produce Factor IX and thus prevent bleeding

Problems with Gene Therapy

- Short lived: hence multiple rounds of therapy would be required (rapidly dividing nature of cells prevents long lasting effects)
- Immune response
- Viral vectors: toxicity, immune responses and inflammatory responses hazards
- Multigene disorders difficult to treat as you would need to introduce more than one gene
- May induce a tumour if integrated in a tumour suppressor gene: insertional mutagenesis

Successful Gene Therapy Trial for Parkinson's Disease

Neurologix (biotech company) has completed Phase I trials of gene therapy for Parkinson's Disease.

- 12 patient study – 4 in each of the three dose escalating cohorts
- Procedures completed under local anesthesia
- All patients discharged within 48 hours
- Patients followed for 12 months
- No adverse events reported

Procedure: an adeno-associated virus vector was used in the trial, and will be used for other first generation products at Neurologix, which will target epilepsy and Huntington's Disease.

Genes get into the brain using liposomes coated in polymerpolyethylene glycol – another potential therapeutic concept for treating Parkinson's. RNA interference or gene silencing using siRNA to degrade RNA of a particular sequence preventing abnormal protein production is a potential therapeutic for Huntington's. As both require the gene in the brain the idea is that tiny liposomes can be created to carry therapeutic DNA through the pores of the nuclear membrane.

Weekly Readings

In gene therapy vectors are used as 'Trojan Horses' to reach required cells, which takes advantage of the millions of years of evolution

Ex vivo gene therapy: target cells are extracted from the patient and the desired gene is inserted into these cells. After transfer the cells are returned to the patient.

In vivo gene therapy: selected genes are delivered directly into the target cells within the whole organism

One of the issues with gene therapy (as discussed in the lecture) is purification, concentration and storage (must be stored at low temperatures). The current vector production systems are unable to quantitatively raise the number of viral particles that are required for a therapeutic application. Some procedures may be used to increase the numbers raised but these have the disadvantages of containing impurities from cell lysates or from pretreatment reagents. Another issue with gene therapy is the immunostability of vectors. Switching serotypes or inducing immunosuppression may potentially combat this.

A crucial factor in the success of gene therapy is targeting. Retrovirus-derived vector are the choice of long-term expression in dividing cells, but have the disadvantage of random integration. A disastrous outcome could occur if this integration activated proto-oncogenes. To limit integration side effects we need to be able to control the site of integration. Hybrid, chimeric vectors may give promise to support long-term gene expression and target specificity.

RNA-guided nucleases known as Cas9 from the microbial adaptive immune system CRIPR (clustered regulatory interspaces short palindromic repeats) is the most rapidly developing programmable nuclease-based genome editing technology, with the potential to directly correct harmful mutations in humans. This system specifically targets any genomic location via a short RNA guide. It recognizes target DNA via Watson-Crick base pairing. CRIPSR loci consist of…

- Clustered set of Cas (CRIPSR-associated) genes
- Series of repeat sequences (direct repeats)
  - Which are interspaced by variable sequences (spacers)
  - These spacers correspond to sequences within foreign genetic elements (protospacers)

An example of how CRISPR could be used is in a disease that results from the duplication of genomic diseases, for example a trinucleotide repeat disorder. This could be treated by inducing two double-strand breaks at the region to excision the duplicated genes.

Challenges: homologous recombination efficiency needs improvement, long-term implications of permanent genome modification remain unclear

Vaccination

Passaging in cell culture: this method develops vaccines through an adaptation to growth on the medium used. Mutants that grow on the medium lose or modify the genes that cause disease and transmission in human hosts. Other adaptations of attenuation include adaptation of viruses to grow at temperatures below the normal body temperature of humans ($37^{o}C$).

Pasteur: formulated the idea of attenuation and demonstrated its utility. He used exposure to oxygen or heat (rabies & anthrax) to attenuate pathogens and create a vaccine.

The origination of in vitro or in habitual host attenuation originated with Calmette and Gurrin, who passaged bovine tuberculosis to develop an attenuated strain for the 'live attenuated bacillus Calmette & Guerin vaccine'.

A revolution for vaccinations was the discovery that cells could be cultured *in vitro* and used as a substrate for viral growth. As you could imagine, vaccine developers took up this method vigorously. A good vaccine should allow for only one replication cycle, maximizing safety. To generate heightened immune responses, adjuvants (such as aluminum salts) are used. Newer adjuvants such as oil-in-water preparations and Toll-like receptor agonists are coming in to use, which give a stronger effect than aluminum salts.

Vaccines may not work the same in all individuals, or in individuals of varying ages. For an example capsular polysaccharide vaccines are not immunogenic in infants because they are unable to mount a B-cell response.

Genetic engineering has paved the way for modern vaccine development. The first vaccine developed with use of gene engineering was for Hepatitis B. Now, many viruses and bacteria are under study as vectors for vaccine antigens. Many attenuated microbes have genes encoding protective antigens from pathogens inserted into their genomes. A vaccine has been produced using genomic analysis to identify proteins that induce bactericidal antibodies with a vesicle to protect against meningococcal group B. This method of vaccine development is called 'reverse vaccinology', where genomic analysis enables the selection of proteins that induce protective immune responses.

The Flu Vaccine

Segments coding for hemagglutinin and neuraminidase are selected and combined with segments coding for the internal genes of well-growing viruses. The vaccine obtained generates functional antibodies against hemagglutinin and neuraminidase, in a safe manner. Most flu vaccines are generated by the growth of viruses in embryonated eggs. After this the whole virus is broken up with detergents and hemagglutinin purifies to serve as the vaccine antigen. Other components of the virus may also be present in the final product.