Antiviral Drugs

- Most infectious diseases in North America are caused by viruses
    - More than 95% of all respiratory disease
- All Class 4 pathogens are viruses
    - Ebola, Marburg, Lassa Fever, Hantavirus, Smallpox

Classification of Pathogens

- Class 1 - No risk or limited risk
    - Works on open lab bench
        - E. coli
    - P1 lab
        - P is level of protection
- Class 2 - Moderate Risk
    - Limited access to lab
    - Lab coat required
    - Laminar hoods provided
        - Herpes virus
    - P2 Lab
- Class 3 - Risk of death or serious illness
    - Restricted access, special training required
    - Surgical gowns, gloves, respirators
    - All liquids/air coming in/out is filtered/treated
    - Everything coming out is autoclaved and incinerated
        - HIV, Y. Pestis
    - P3
- Class 4 - Lethal, highly infectious, untreatable
    - Lab accessed by airlock, special training required
    - “Space suit” worn, shower going in or out
    - Low pressure in lab (leaks stay in), airlock
    - All liquids and gases filtered/treated going in and out
        - Ebola, Marburg, Lassa fever, Hantavirus, smallpox
    - P4 lab

Virus Structure

- All viruses categorized as having
    - Genetic information
        - DNA or RNA
    - Surrounded by a capsid
        - Hollow container made of protein
- Some viruses carry additional proteins
    - Enzymes
    - Regulatory proteins
- Some Viruses are Enveloped
    - Capsid is surrounded by a membrane
    - Remnant of the host cell membrane

    - Contains viral proteins

Self-replicating Piece of Cellular Machinery

- Many viral proteins are structurally related to host proteins
    - Oncogenes
- Viral proteins often bind to host protein and alter that protein's function
    - Control cellular regulatory systems
- Common Elements (all viruses)
    - Duplications of genetic information
    - Productions of viral protein
- Viral processes utilize host proteins and machinery
    - Ribosome
    - Nucleic acid polymerases
- Viruses are not living

West Nile Virus

- 3 diff proteins in outside of structure
- 5 fold symmetry

Tobacco Mosaic Virus

- Red spiral - nucleic acid
- Blue spiral - protein

Difficulties in Developing Antiviral Drugs

- Each virus is unique
    - Each virus requires different drug
- Most viral proteins act by binding to host proteins
    - This is very difficult to target with drugs
    - Proteins tend to bind to each other very tightly
    - Utilize large contact surfaces
        - Creates tight binding between viruses
        - In order to break this tight bonding, we must develop a large drug which causes difficulties
    - Need to avoid interfering with normal host cells

Cell Signaling Systems use Protein Binding

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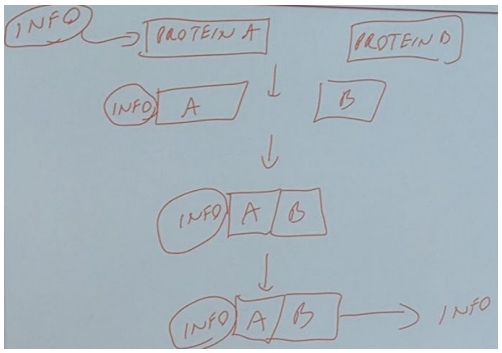
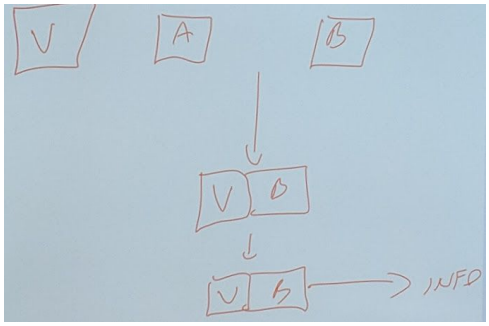
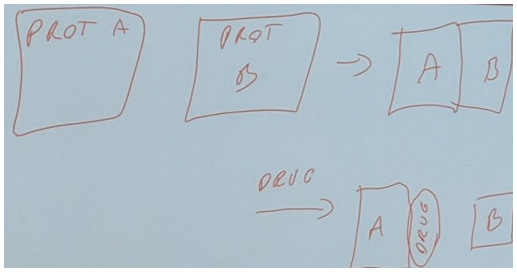


Many Viral Proteins Operate by Binding to Host Proteins

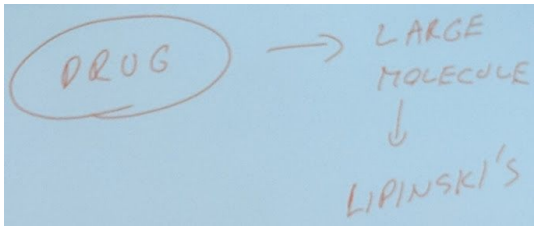
- 

- V - viral protein

Can Block Protein Binding in the Lab

- 

Protein Binding is Difficult to Control with Drugs

- 

Viral Enzymes as Drug Targets

- Viruses carry very few enzymes
    - Typical virus has 1 or 2
- Most involve nucleic acid replication
    - Host cells also make nucleic acids
    - Substrates, mechanisms, and structures are similar
- How do you block viral enzyme without blocking host enzyme?
    - Fundamental problem in dev. Antiviral drugs

Limited Number of Targets

- Most viruses have small genomes
    - As few as 10 genes
    - On average, 1 or 2 are enzymes
        - Most involve nucleic acids (host selectivity problem)

Today only a Few Good Antiviral Drugs Exist

- Hepatitis C (cure)
- Herpes (treat)

- HIV (manage)

Major Problems with Antiviral Drugs

- Selectivity
    - Kill virus without killing host
- Diagnosis
    - Drugs specific for each virus
    - Many viruses produce similar symptoms
        - More than 200 viruses cause colds
    - Only way to know for sure is biochemical test
- Resistance
    - Mutation rates in viruses very high
    - Viruses quickly develop resistance to drugs (days or weeks)

Immunization has been Very Successful for Viruses

- All time greatest achievement of medicine
    - Smallpox (disease eliminated)
    - Polio (Disease almost eliminated)
        - 1950 - hundreds of millions of cases (world)
        - 2018 (33 cases worldwide)
    - Measles
    - Mumps

Virus Life Cycle

- <u>Absorption and penetration</u>
    - Virus bInd to host proteins on outside of host membrane
        - Capsid binds directly and is passed inside before opening
        - Envelope fuses with host cell membrane releasing capsid inside the cell
    - Genetic info is injected into the cell
        - Virus may also inject viral protein (enzyme or regulatory proteins)
    - Absorption and penetration are poor drug targets
        - Binding involves protein-protein interactions
            - Protein surface areas are very large
            - Difficult to inhibit protein-protein binding with small molecules
        - Limited success in HIV
            Fuzeon - peptide with 36 amino acids
        - Maraviroc - small molecule
- <u>Capsid Opens Releasing Contents</u>
    - Release of viral nucleic acid
    - Capside open releasing genetic information into the cell
        - Difficult to target
            - Protein interactions, pH changes
        - Two successful drugs
            - Influenza
            - Block an ion channel
- <u>Synthesis of Regulatory Proteins</u>

        - Viral proteins are made
            - "Take over" normal cells systems
                - Nucleic acid replication
                - Expression of viral protein
                - Suppression of host cell defenses (apoptosis)
                - Binding to host proteins
            - No drugs currently exist for this phase
    - Synthesis of RNA or DNA
        - Viral genome is replicated using host enzymes
        - Some viruses have their own enzymes for this (drug targets)
        - Most antiviral drugs target this phase
            - Require unique viral enzyme
            - Prevent viral nucleic acid synthesis
    - Synthesis of Structural Proteins
        - Utilize the host Ribosome
            - Poor drug target
        - Some viruses utilize specific enzymes for protein maturation
            - Protease Drugs
                - HIV, Hepatitis C
    - Assembly of Viral Particles & Release from Host Cell
        - Capsid proteins self-assemble
            - Nucleic acid inside
            - Viral proteins outside
        - Release may destroy cell
            - Lyric virus, herpes, influenza
        - Cell may remain intact
            - Papilloma, herpes
        - Only a few drug targets (HIV, Influenza)

Antiviral Drugs require Viral Enzyme Targets

- Enzyme should be structurally unrelated to host enzymes
    - Provides selectivity
- Most viral enzymes are involved in nucleic acid replication

Herpes

- Virus causes chronic recurrent infections
- Virus is able to escape immune system
    - Latency in neurons
- HSV-1
- HSV-2
    - Other subtypes less prevalent

HSV-1

- Cold sores and fever blisters on the mouth and nose, sometimes eye
- More than 80% of the population is infected
- Only 10-20% experience outbreaks

- Virus escapes eradication by latency inside neurons
    - Inaccessible to immune system
    - Stress, sunlight, immune suppression trigger outbreaks
    - Virus travels down axon to epithelial cells
- Lytic Infection
    - Only in epithelial cells, does not damage neurons
- Outbreaks
    - Lytic infection
        - Viral activity is short (less than 24 hours)
        - Viral damage is minimal
        - Most damage is caused by the immune system
            - Over stimulated and destroys most of the tissue
            - Drug must be administered quickly

HSV-2

- Sores and fever blisters on the anus and genitals
- Infects 15-20% of population
- Infection is much more violent than HSV-1
- Usually one outbreak per year
- Most common type of STD
- Viral activity is short
    - Viral damage is minimal
- Most damage by immune system
    - Drug must be administered quickly

Herpes Virus Structure

- Genetic Information is double stranded DNA
- Very complex virus
    - More than 70 genes
    - Most viruses have less than 10
- Virus has its own polymerase
    - Enzyme that can make nucleic acid

Components of Nucleic Acid

- Nucleosides (no phosphate)
- Sugar (2-deoxyribose on DNA or ribose in RNA)
- Base - nitrogen containing aromatic heterocycle
    - Heterocycles are ring structures incorporating heteroatoms

Base Form a Type of Alphabet

- Four different bases (ACTG)
- Each base is “recognized” by other molecules because of its shape and hydrogen bonding pattern
- Enables binding of only certain structures to each base

Structure of Nucleic Acids

- Polymers formed by connecting nucleosides together using phosphate esters
- The individual units are now called nucleotides (phosphate containing)

- Forms a long strand
- Backbone of sugars and phosphates
- One base per sugar
- Bound in antiparallel direction
    - Stabilizes structure

Nucleic Acid Strand

- Nucleic acid encodes information in its structure
- Info is stored as the sequence of bases
- Polymerases use one strand as a template to make another strand

Nucleic Acids can form Double Strands

- Stabilize the molecules
    - Long-term storage
- Second strand provides an easy way to replicate or read information
- Error checking

Polymerases copy Nucleic Acids

- Use one strand as a template to make another strand
- Nucleotides are added one at a time, matching each added base against its complement on the other strand

Polymerase creates new Strand

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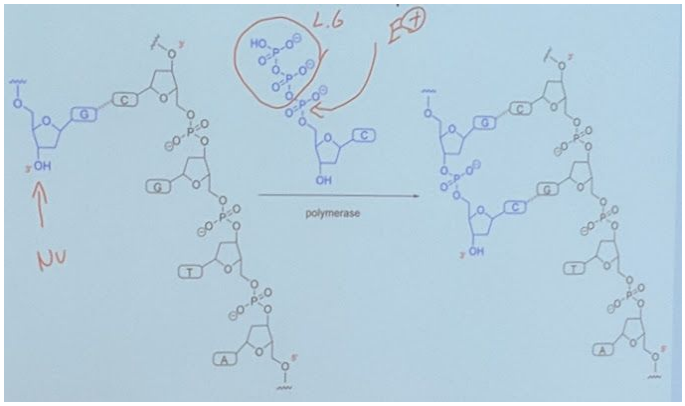

Polymerase adds Bases One-at-a-time

- After each base is added, a new 3' OH nucleophile is available for next addition

Rational Drug Design

- Use knowledge of enzyme mechanism and substrate to design a drug

Selectivity Problem with Nucleic Acids

- Must block viral enzyme without blocking host
- No chemical difference between viral nucleic acid and host nucleic acid
- Must find a drug that block viral polymerase but does not bother host polymerase

Poor Selectivity = Toxicity

- Interference with normal cell function creates problems
- Viral polymerase creates a viral nucleic acids which creates a viral protein

Strategy 1 - Non-Natural Base

- Resulting nucleic acid strand is non readable by host enzymes

Effects of Non-Natural Base

- Substrate structure disrupted, polymerase cannot function
- Nucleic acid not readable by other enzymes

Requirements for Non-Natural Base

- Drug is a substrate for kinase
    - Host
    - Virus (rare)
- Drug is a substrate for viral polymerase
    - Gets incorporated into viral nucleic acid
    - Creates an “unreadable” strand
- Drug must NOT be a substrate for host polymerase
    - Will cause side effects

Selectivity Requirements for Non-Natural Base

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