Basic Virology

- 1. Consist of a genome, ***either*** RNA or DNA, that is surrounded by a protective protein shell (capsid). This shell often is surrounded by an envelope that consists of protein and lipid.
- 2. They multiply only in living cells. Totally dependent upon host cell organelles and energy. Parasites at genetic level.

Origin of Viruses

- 1. Viruses are the products of regressive evolution of free-living cells (like mitochondria which have their own genetic info and replicate on their own).
- 2. Viruses are derived from cellular genetic material that has acquired the capacity to exist and function independently. *{this is most likely except for Pox Viruses}*

Handling of Viruses: Culture Systems

- Viruses can be seen only by EM, and this requires in excess of 10^{11} particles. Viruses usually detected by indirect means.
  - Multiplication in suitable culture system and detection by effects that it causes
  - serology- use of specific antibodies
  - detection of viral nucleic acid

Detection of animal Viruses

- 1. Recognized by the manifestation of some abnormality in host organisms or host cells. Symptoms can vary from inapparent infections (detectable by Ab formation), to development of local lesions or mild disease to severe disease and death.
- 2. In cells, the symptoms vary from from changes in morphology and growth patterns to cytopathic effects (rounding, breakdown of organelles, etc..).

Characteristics of cultured cells

- 1. Understanding of how viruses affect cells can only come from the study of cloned cells, cultured and infected in vitro.
- 2. Whole animal cultures, Organ cultures, Cell cultures, fertilized chick eggs.
- 3. Many, but not all, cells can be cultured *in vitro*.
  - Single cell suspension (trypsin tx)
  - cells attach and multiply in appropriate cell culture medium

Cell Cultures

- Cultured cells either diploid or heteroploid (usually having more than normal number of chromosomes). Heteroploid cells have advantage of being continuous (continuous cell lines).
  - Sources are:
    - kidney
    - fibroblast
    - whole body homogenization
    - tumor cells
    - embryos

Cell Culture Medium

- 1. Need physiological concentrations of the 13 essential AA's, vitamins, salts, glucose, and a buffering system that generally consists of bicarbonate in an atmosphere containing about 5% CO_2.
- 2. Need also to supply serum (about 5-10%), that is not predicated on cells species (usually calf or fetal bovine).
- 3. Use of antibiotics.

Cell Growth

- 1. When cell grow they attach onto plate surface and flatten. The only time they are not fully extended is when they are going through mitosis (they become rounded).
- 2. When they become confluent they stop growing (unless transformed). *(Contact inhibition*)
- 3. Two types of animal cells generally used: epithelial and fibroblast grow best in vitro.

Cell Growth

- 1. Primary cultures: first cultures after tissue dispersion. When they reach confluence they are treated with trypsin and passaged to form secondary cultures. This can occur for about 60 doublings unless cells are transformed. Mutations occur at high rates and cells of secondary cultures may not be "the same" as the primary culture cells, and cells isolated in one lab may be different from cells isolated in another lab(and respond differently)

Cell Growth: Multiplication Cycle

- 1. The interval between successive mitoses is divided into three periods: (a) G1: precedes DNA replication; (b) S: DNA replicates; c) G2 during which the cell prepares for the next mitosis.
- 2. RNA and protein are not synthesized while mitosis proceeds-- during metaphase-- but are syn during the rest of the cycle.
- 3. Non-growing cells arrested in G1 (resting state referred to as G0).

Other Indirect Methods for Viral Identification

- Hemagglutination & Hemagglutination Inhibition
  - some viruses attach to surface receptors on RBC's. This will cause agglutination. The viral load can be quantitated by diluting virus and observing where agglutination stops
  - test is quick (30 minutes), but insensitive (takes 106 PFU (plaque-forming units) to get detectable agglutination.
- Inhibition- addition of Ab's to check for inhibition of agglutination and for presence of Ab's in subject.

Hemagglutination

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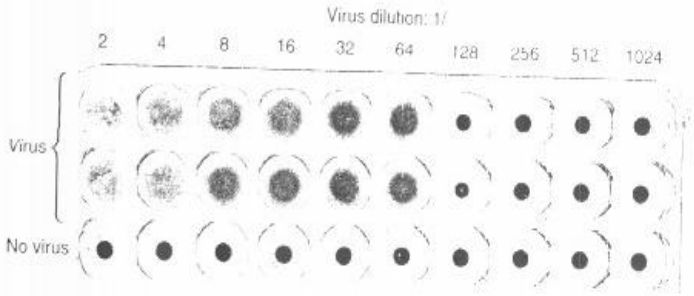
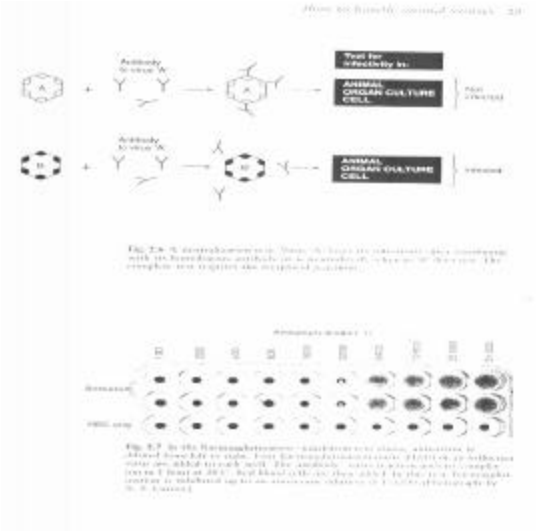


Fig. 2.5 Haemagglutination titration. Here an influenza virus is serially diluted (from left to right) in depressions in a plastic plate. Red blood cells (0.5%, v/v) are then added and mixed with each dilution of virus. Where there is little virus, cells settle to a button (from 1/128) indistinguishable from those to which no virus was added (row 3). Where sufficient virus is present (up to 1/64), cells agglutinate and settle in a diffuse pattern. (Photograph by A. S. Carver.)

Hemagglutination Inhibition



Isolation of Animal Viruses

- 1. Source may be excreted, secrete, blood, or tissue.
- 2. Unless process immediately specimen is stored at -70°C (temp of dry ice).
- 3. Suspension is made by homogenization or sonication, and centrifuges to remove large debris.
- 4. Tested for presence of virus by injecting into test species to cause same effect (Koch's Postulates). Usually newborn animals or cells.

Adaptation and Virulence

- 1. Adaptation: during isolation there may emerge variants capable of growing more efficiently in host cells. This is adaptation.
- 2. Often these variants damage the host less severely than the wild-type and are therefore said to be less virulent.
- 3. This comes in handy in making attenuated viral strains through repeated passages in tissue culture.

Measurement of Animal Viruses

- May be measured as infectious units (ability to infect, multiply, and produce progeny) or as virus particles (irrespective of their function as infectious agents).
- 1. Infectious Units: measurement of the amount of virus in terms of infectious units per unit volume= titration. To measure the titer of a virus suspension you must infect the host or target cells in such a way that each particle that causes a productive infection elicits a recognizable response.

Infectious Units

- 1. Plaque Formation:
  - monolayers of susceptible cells are inoculated with small aliquots of serial dilutions of virus suspensions. Viral progeny are made and infect adjacent cells and this is repeated during the 2-12 day incubation until visible "*plaques*", holes, are seen in monolayer. Each plaque is caused by a single virus (if diluted properly) (PFU).
  - Cheap, simple, but not all viruses cause CPE.

Measurement of Viruses

- Focus Formation:
  - Many tumor viruses do not destroy the cells in which they multiply, and therefore do not produce plaques. Instead they cause cells to change morphology and to multiply at a faster rate than uninfected cells. These are called "*transformed cells*". Colonies of transformed cells may develop into FOCI that are large enough to see with naked eye (FFU=focus forming units)

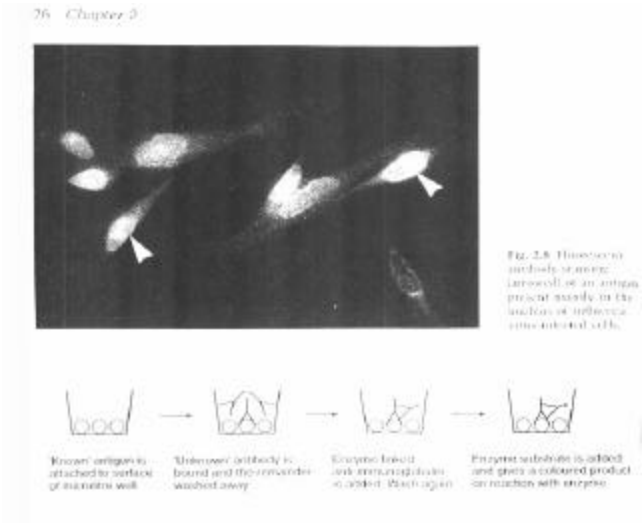
Hemagglutination Assay

- Enumeration of total number of virus particles irrespective of function:
- 1. Many animal viruses adsorb to the RBC's of various animal species. Each virus particle is multivalent, or can bind to more than one cell at a time. Usually virus can only bind to two RBC's since RBC is so much larger. In a RBC:virus mixture where the number of virus particles exceeds the number of RBC's, a lattice is formed and RBC's agglutinate. Unagglutinated cells form a dark button, while agglutinated cells do not. Can determine viral concentration, by titration of virus, because is takes just slightly more than the number of cells to effectively agglutinate RBC's. Very effective to enumerate myxoviruses (i.e., influenza).

Serology

- Viral presence detected by use of antibodies against specific viral antigens.
  - When virus infects cells an immune response arises and antibodies made. Can detect presence of Ab's in serum
  - Can detect specific viral antigens in serum
    - Detection by:
      - ELISA
      - Western Blots
      - Fluorescence
      - Inhibition of action (neutralization/blocking)

ELISA & Fluorescent Staining



Viral Structure

- Viruses consist of nucleic acid and protein. The NA is the genome that contains the information necessary for virus multiplication; the protein is arranged around the genome in the form of a shell that is the *capsid*. The shell plus the NA is the *nucleocapsid*. Some virus particles consist of a naked nucleocapsid, many others posses an *envelope* that is acquired from the host as the virus buds out. The complete particle is the *Virion*.

Structure

- Capsid: composed of repeating subunits, identical or belonging to only a few different proteins. The simplest are composed of a single protein molecule; more complex units are composed of many subunits of either identical of different protein molecules that are called *capsomers*. The function of the capsid is to protect genomes and to help get genome into host cells

Structure

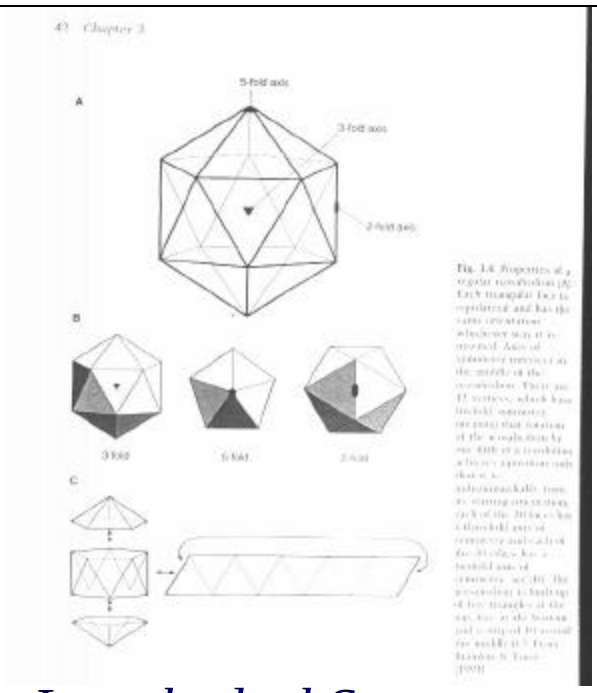
- Envelopes: only 7 families of animals viruses exist as naked nucleocapsids, all the others are enclosed by lipid envelopes that are acquired by the budding of viruses through the host cell membrane. These may be virus modified to contain viral proteins (spikes), but also have host cell components. Envelope helps in attachment to host cells. There is a virus specific matrix protein between the nucleocapsid and the envelope.

Nucleocapsid morphology

- **Helical symmetry**: extended NA (tobacco mosaic virus: TMV). (also ortho-, myxoviruses, rhabdoviruses, arenaviruses and coronaviruses). The capsid proteins of these viruses are arranged in a helical manner. The length of the helical capsid is dependent upon the length of the nucleic acid that is associated within the capsid. (a ratio of about one protein molecule for every 3 ribo- nucleotides in TMV)
- Length of helical capsid is dependent upon the length of the nucleic acid

Structure of Spherical Viruses

- Subunits arranged around the vertices or faces of an object with cubic symmetry (i.e., tetrahedron, cube, octahedron, dodecahedron {12 regular pentagons}, or icosahedron {constructed from 20 equilateral triangles}).
- An icosahedron is made up of 20 triangular faces, five at the top, five at the bottom, and ten around the middle. An icosahedron has three axes of symmetry: fivefold, three fold, and twofold.



Icosahedral Symmetry

Nucleocapsid morphology

- Icosahedral symmetry: capsid consists of a shell of protein molecules (protomers) that are clustered into small groups called capsomers (bonds between molecules within a capsomer are stronger than bonds between capsomers). Capsomers can be composed of identical or different protomers (usually not more than 3).
- X-ray diffraction shows that capsomers arranged precisely according to icosahedral patterns characterized by 5:3:2-fold rotational symmetry.

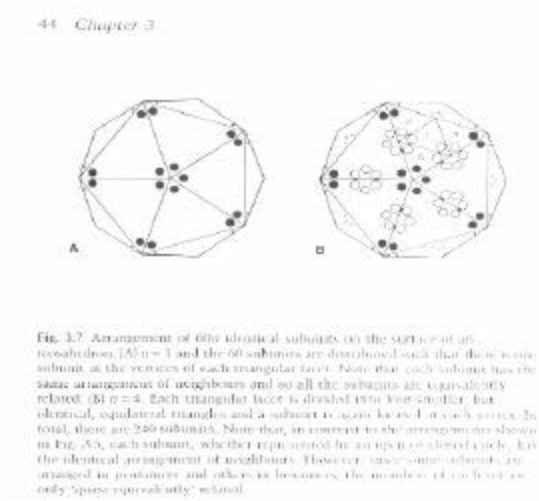
5:3:2-Fold Rotational Symmetry

- 5 Fold (5 X) rotational symmetry is viewed by passing an imaginary axis through the vertices of the icosahedral triangular faces, and viewed from the top and bottom of the virion, one can envision 5 triangular faces.
- 3 Fold rotational symmetry is viewed when the rotational axes pass through the centers of the triangular faces.
- 2 Fold symmetry occurs when the axes pass through the edges of the triangular faces.
- The icosahedron possess 12 vertices, 20 triangular faces, and 30 edges.

Triangulation of Spheres

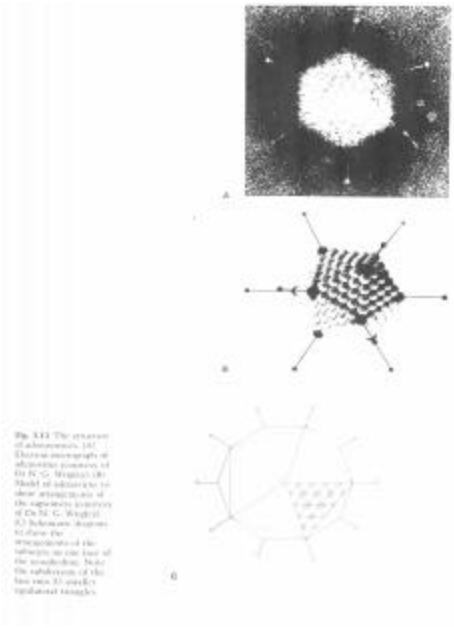
- There are 12 vertices in an icosahedron. There thus are 12 groups of five subunits (**pentons**).
- If each triangular face is further subdivided into four smaller and identical equilateral triangles, the vertices of these smaller triangles will be composed of rings of six subunits (**hexamers**).
- To calculate the total number of capsomers covering the icosahedron use: $10(n-1)^2+2$ (n is the number of capsomers along one edge).

Pentons and Hexons in Icosahedron



Icosahedral Symmetry

- The **adenovirus** capsid contains an icosahedron with six capsomers (2 at the vertices and 4 along the edge) along each edge and 252 capsomers total. Of these, 240 are spherical and are situated along the edges and on the faces of the icosahedron: each has 6 nearest neighbors and is known as a hexon. The remaining 12 are situated at the 12 vertices of the icosahedron and have 5 nearest neighbors, and these are known as pentons. The pentons have a highly characteristic shape, with a spherical base and a long fiber that helps in attachment



Other Icosahedral Viruses

- Picornavirus: 60 identical capsomers, each composed of four different proteins, each located equidistantly from a common center, which results in a spherical capsid. These capsomers are bonded into groups of 5 (pentons at vertices), 12 groups making up the capsid.
- Papovavirus: consists of 72 identical pentameric capsids.

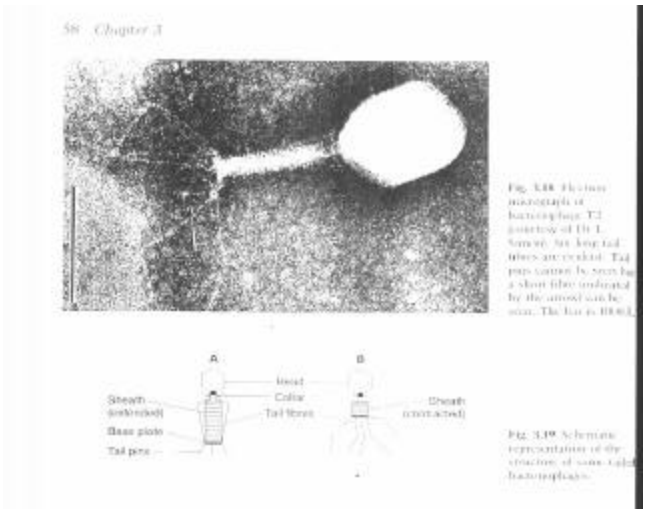
Reoviruses

- Unique in that they possess two capsid shells. Both possess icosahedral symmetry, but so far technology has not been able to determine the total number of capsomers or the precise manner in which they are arranged.

Structure of Retroviruses

- More complex structure: they are composed of a coiled nucleocapsid located within a shell that possesses icosahedral symmetry, and is surrounded by an envelope with glycoprotein spikes. They consist of 2 identical strands of NA (RNA). They also contain an enzyme unique to the retrovirus family, reverse transcriptase.

Structure of Bacteriophages



Relative Sizes of some Important Viruses: *(mitochondria= 1 um, diameter of animal cell= 0.75 m, length of DNA in poxvirus= 7.5 m)*

- **DNA Viruses**
  - Parvovirus= 22 nm
  - Papovavirus= 55 nm
  - Adenovirus= 75 nm
  - Herpesvirus= 100 nm
  - Poxvirus= 250 X 300 nm
- RNA Viruses
  - Picornavirus= 28 nm (ribosome)
  - Togavirus= 40-50 nm
  - Reovirus= 75 nm
  - Influenza virus= 80-120 nm
  - Paramyxovirus= 150 nm
  - Rhabdovirus= 180 X 75 nm (bullet shaped)

Viral Nucleic Acids

- DNA vs RNA (one or the other, not both)
- single vs double stranded (DNA or RNA)
- linear vs circular
- plus polarity vs minus polarity (in RNA viruses)-- the + strand can bind directly to ribosome, but the - strand needs to make + before protein synthesis occurs.
- Hepatitis B has smallest genome size, while the herpes- and pox viruses have largest
- Segmentation- Reoviruses (10), influenza (8), etc...

Viral Nucleic Acids

- Heat to denature. Temperature to melt DNA dependent upon ionic strength of bonds and on the guanine:cytosine content (higher %GC higher temp needed). When cooled below 25°C the strands reanneal. cRNA will hybridize with complementary strands of DNA.
- Use of CsCl gradients (buoyant density).
- Endo- and exonuclease treatment

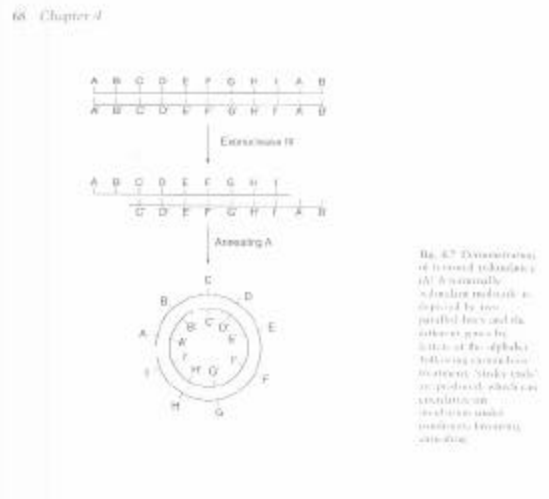
Unique Features of Viral Nucleic Acids

- Many viral nucleic acids also show:
- Terminal redundancy-- A,B,C,…..X,Y,Z,A
  - Can show this by treatment with lambda exonuclease (disgests from the 5' end) melting and annealing and getting a circular molecule.
  - Both herpesvirus DNA and retrovirus RNA possess repeated sequences that are about 400 residues long.
- Cross-linking-- DNA of poxviruses are unique in being cross-linked at their ends. Shown by melting and annealing to get a circle 2 X as large as original.

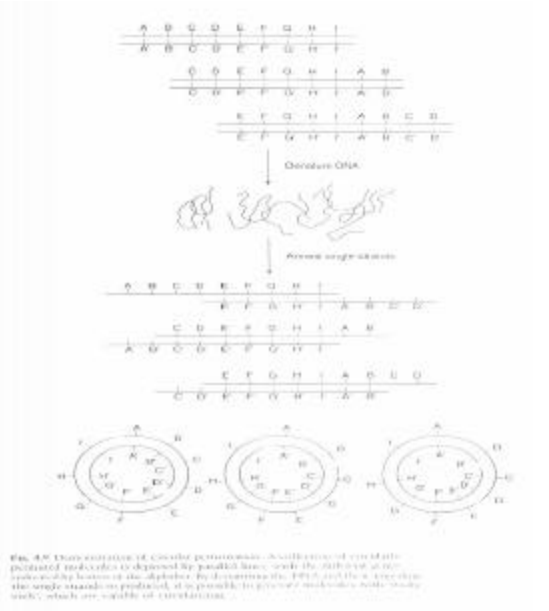
Unique Features of Viral Nucleic Acids

- Circular Permutation: Bacteriophage T4 has a linear DNA molecule but its genetic map is circular. If the genome is placed around a circle and the circle is broken at any point a collection of linear molecules is obtained with the order of genes unchanged. If these are denatured and reannealed complementary strands will come together with sticky ends which enable the molecule to circularize.
- Covalent linkage with protein-- RNA of picornavirus (ss, linear) is linked at its 5'-terminus to the tyrosine residue of a 22 AA long protein, and each double stranded DNA of adenovirus (linear) is linked to a protein with MW 55,000. May function as primers in the replication of these NA's.

Terminal Redundancy



Circular Permutation



Unusual Bases in Nucleic Acids

- DNA from certain bacteriophages has thymine replaced by uracil (bacteriophage PBS1) or hydroxymethyluracil (SP8)
- Cytosine replaced by hydroxymethylcytosine (bacteriophages T2, T4, T6)
- In T-even phages hydroxymethylcytosine may be further substituted with glucose or gentiobiose
- These changes specified by virus.
- Methylation patterns are different in viral DNA than in host DNA.
- Presence of overlapping genes

Importance of Differences in NA

- Presence of unusual bases may help virus to subvert cell's biosynthetic machinery and redirect it to the production of new virus
- Important in replicative process and allows differentiation from host cell
- These differences serve as potential points for attack on the virus specifically (i.e., protease inhibitors and AZT in retroviruses)

Infectivity of Viral Nucleic Acids

- 1952-- Hershey & Chase found that introduction of bacteriophage DNA into bacterial cells resulted in the formation of progeny virus particles
- 1956-- Gierer & Schramm showed same thing happened when RNA extracted from TMV
- Naked NA's how low infectivity (degraded by nucleases, taken up poorly) Host range broader!
- NA's that require viral enzymes (retroviruses, -stranded RNA viruses) are not infectious.

Viral Proteins

- Proteins (structural proteins-- coded for by viral genome)
  - sole component of the capsid
  - major component of the envelope
  - associated with nucleic acids as internal core proteins
  - vary in size (10,000 --> 150,000)
  - vary in number (3 to > 50 in different viruses) [identify by PAGE-SDS]

Viral Glycoproteins

- Form spikes or projections from envelope
  - hemagglutinin & neuraminidase of influenza
  - consist of about 10-15 monosaccharide units
  - specificity of sequence due to glycosyl transferases (assemble from monosaccharide units) as well as the nature of protein(i.e., viral proteins differ from cellular)

Proteins with specialized functions

- Hemagglutinins-- both naked and enveloped have these; agglutinate RBC's. Found on ortho- & paramyxoviruses
- Neuraminidase-- hydrolyzes the galactose-N-acetylneuraminic acid bond at end of oligosaccharide chains and liberates N-acetylneuraminic acid. Helps in the release of viral particles from the cells in which they were made.
  - Orthomyxoviruses have one spike of H and one of N
  - Paramyxoviruses have two spikes, one of which has both H and N activities (the other only H)

Proteins with specialized functions

- RNA Polymerases-- many viruses need these if they have RNA with negative polarity, if they are double stranded (RNA), or DNA viruses
  - Two sources
    - use host RNA polymerase (DNA viruses replicate in nucleus)
    - carry own RNA polymerase (RNA or DNA (pox) viruses that replicate in cytoplasm)
- RNA dependent DNA Polymerase-- (RT)

Viral Lipids

- Composition usually resembles the membranes of the host (same virus grown in different cell strains may have different lipid composition)
- obtained by budding through host membrane
- may have viral specific proteins and glycoproteins as spikes and receptors

Classification of viruses

- Viruses that are recognizably different in more than one gene are designated as a *species*
- species that exhibit genetic similarity are grouped together into *genera*
- genera are grouped into *families* based on morphology, the physical and chemical nature of viral components and on molecular strategies to express themselves and to replicate

Baltimore Classification

- Class I: consists of all viruses that have ds DNA
- Class II: **(a)** consists of viruses that have ss DNA of the same sense as mRNA (I.e., + polarity)
- Class II: **(b)** consists of viruses that have DNA complementary to mRNA
- Class III: consists of viruses that have a ds RNA. All known viruses have segmented genomes, but mRNA synthesized from only one strand of each segment
- Class IV: ssRNA viruses with + polarity
- Class V: viruses that have ssRNA with - polarity
- Class VI: ssRNA viruses that have DNA intermediate during replication

Major Families of Animal Viruses : DNA- Containing

- Poxviridae: brickshaped, 225 X 300 nm, multiply in cytoplasm
  - Variola major-- smallpox (humans)
  - Vaccinia-- cowpox (cattle)
  - Orf-- pustular dermititis (sheep, goats, humans)
- Herpesviridae: Enveloped, icosahedral, 180-250 nm
  - Herpes simplex type 1 -- skin, facial (humans)
  - Herpes simplex type 2 -- genital (humans)
  - varicella-zoster-- chickenpox (humans)
- Cytomegaloviruses: jaundice, hepatosplenomegaly
- Epstein-Barr virus: mononucleosis (humans)

Major Families of Animal Viruses : DNA- Containing

- Adenoviridae: naked, icosahedral, 70 nm, 41 human serotypes grouped into 7 sub-groups based an antigenicity. Some cause tumors in hamsters, others transform cells. No tumors in humans? Mild diseases.
  - Cause respiratory diseases
  - keratoconjunctivitis
  - gastrointestinal diseases in children

Major Families of Animal Viruses : DNA- Containing

- Papovaviridae: naked, icosahedral, 55 nm, 2 genera based on size: papilloma- (larger) and polyomaviruses
  - Human papilloma viruses (HPV)-- warts, genital tract cancers, cervical cancer
  - Polyoma virus (mouse), Simian vacuolating agent (SV40) (monkey, human) & JC virus (human) [JC causes progressive multifocal leukoencephalopathy (PML)]

Major Families of Animal Viruses : DNA- Containing

- Hepadnaviridae: enveloped, icosahedral, 42 nm, smallest human or animal genome known (about 3 kb). Their lipoprotein envelope causes self-association forming *Dane Particles*, found in sera. Replication of hepadnavirus DNA involves reverse transcription of RNA into DNA.
  - Hepatitis B Virus (HBV)- acute & chronic hepatitis, cirrhosis, hepatocarcinoma

Major Families of Animal Viruses : DNA- Containing

- Parvoviridae: naked, icosdahedral, 22 nm, contain ss DNA. Certain members can encapsidate either + or - DNA strand. Often found with tumors because they need some sort of factor to replicate that is supplied by growing cells
  - Feline panleukopenia virus (FPLV)-- no known symptoms in humans

Major Families of Animal Viruses : RNA- Containing

- Picornaviridae-- naked, icosahedral, 25-30 nm
  - 4 Genera- 2 are acid stable and 2 are acid labile
    - Enterovirus- (acid stable)
      - Polio Virus (3 serotypes)- Human & monkey-- Poliomyelitis
      - Coxsackie Virus A (23 serotypes)- Human & monkey-- striated muscle damage, common cold symptom, aseptic meningitis, paralysis
      - Coxsackie Virus B (6 serotypes)- humans & mouse-- fatty tissue and CNS damage, severe systemic illness of newborns
      - ECHO (enteric cytopathogenic human orphan) (32 serotypes)- humans-- paralysis, diarrhea, aseptic meningitis

Major Families of Animal Viruses : RNA- Containing

- Cardiovirus- (acid stable)
  - encephalomyocarditis virus (EMC)-- mouse and humans-- mild febrile illness
- Rhinovirus- (acid labile)- (113 serotypes)
  - human rhinovirus- humans-- common cold, bronchitis, croup
- Apthovirus- (acid labile)
  - foot and mouth disease virus (FMDV)- cattle, swine, sheep, goats

Major Families of Animal Viruses : RNA- Containing

- Togaviridae- enveloped, icosahedral, 60-70 nm.

Includes many plus-stranded RNA viruses. 4 genera, of which the Alphaviruses is the largest (also rubivirus, pestivirus and arterivirus). Includes the Arboviruses (arthropod-borne). Multiply in bloodsucking insects as well as in vertebrates and alternate between an insect vector (mosquito or tick) and a vertebrate reservoir. Rarely produces disease in either. Many cause sub-clinical disease in humans, and some cause severe disease and death. Commonly named for geographic site where isolated.

Major Families of Animal Viruses : RNA- Containing

Disease	*Reservoir*	*Symptoms*
Eastern equine encephalitis	Birds	encephalitis (freq)
Semliki forest virus	Monkeys	encephalitis (rare)
Sindbis	Monkeys	fever, rash, arthritis
Venezulean equine enceph	Rodents	encephalitis

- Genus Rubivirus
  - Rubella virus- humans, severe deformities of fetuses in 1st trimester of pregnancy

Major Families of Animal Viruses : RNA- Containing

- Flaviviridae- enveloped, icosahedral, 45-55 nm. *Flavus* (yellow). Comprises old group B arboviruses. Put into own family because it was found to have different replication strategy. Some mosquito borne and others tick borne.
  - Mosquito borne-
    - Yellow fever virus (reservoir=monkey)- hemorrhagic fever, hepatitis
    - Dengue Virus (4 serotypes)- (reservoir=humans)- fever, arthralgia, rash

Major Families of Animal Viruses : RNA- Containing

- Cornaviridae-- enveloped, helical, 120 nm. Up to 20 nm long petal shaped spikes ("peplomers") attached to envelope. **Contains plus stranded DNA**.causes respiratory, enteric and neurologic infections, hepatitis, nephritis, pancreatitis. Family is separated into antigenic groups, not genera. Human coronaviruses grow well in cultured cells only after extensive adaptation by passage. Two best studied strains are 229E and OC43. (no examples)

Major Families of Animal Viruses : RNA- Containing

- Reoviridae- naked capsids that possess 2 capsid shells with icosahedral symmetry, 75 nm. [respiratory-entero] Genome consists of 10, 11 or 12 segments of ds RNA. 6 genera with different hosts and morphologies.
  - Orthoreovirus- mammalian & avian
  - orbivirus- bluetongue (sheep), encephalitis
  - phytoreovirus-rice dwarf (plants)
  - rotavirus- human rotavirus-acute infantile gastroenteritis
  - cypovirus- silkworm, lepidoptera
  - fijivirus- maize rough dwarf virus- plants, leaf hoppers

Major Families of Animal Viruses : RNA- Containing

- Birnaviridae- naked, icosahedral, 60nm. Family of viruses that contain ds RNA with only two genome segments, one of which encodes the RNA-dependent RNA polymerase, and the other several structural proteins. No human pathogens.
- Rhabdoviridae- bullet-shaped, enveloped, helical, 180 X75 nm
  - rabies virus- host=all warm blooded animals/encephalitis
  - vesicular stomatitis virus (VSV)- cattle, horse, swine. No human symptoms.

Major Families of Animal Viruses : RNA- Containing

- Filoviridae- enveloped, helical. Particles exist as filaments with uniform diameter (80 nm) but variable length (up to 14,000 nm). Pleomorphic, branched, circular and U-shaped. Only two viruses in family and both are lethal to humans, and can be studied only under stringent conditions.
  - Marburg virus- host = humans, acute hemorrhagic fever, frequently fatal
  - Ebola virus- (same as above)

Major Families of Animal Viruses : RNA- Containing

- Paramyxoviridae- enveloped, helical, 150 nm.
  Used to be part of the orthomyxoviruses, but these genomes are NOT segmented, and they employ a different strategy in genome expression and and gene replication. Possess two types of glycoprotein spikes, but one has both hemagglutinin and neuraminidase activities and the other possesses membrane-fusing activity.
- Genus Paramyxovirus
  - Sendai virus- human/pig/mouse; croup, common cold
  - mumps virus- human; parotitis, orchitis, meningoencephalitis
- Genus Morbillivirus
  - Measles- humans; measles, chronic degeneration of the CNS (SSPE)

Major Families of Animal Viruses : RNA- Containing

- Orthomyxoviridae- enveloped, helical, 80-120nm.
  The term myxovirus coined to denote the unique affinity of influenza viruses for glycoproteins (hemagglutinin and neuraminidase). Influenza A and B have these spikes, but Influenza C has only one type of glycoprotein spike which is a hemagglutinin and binds to different receptors. There are 13 serotypes of H and 9 serotypes of N. Genome is **segmented** (7 segments)
  - Influenza A- human; acute respiratory disease. 4 subtypes
  - Swine influenza virus
  - Avian
  - Influenza B- (4 subtypes)
  - Influenza C

Major Families of Animal Viruses : RNA- Containing

- Retroviridae (RNA tumor viruses)- enveloped, icosahedral, 100 nm, coiled nucleocapsid. Large group of viruses characterized by common morphology. Genome consists of two identical plus-stranded RNA molecules, and possession of *Reverse Transcriptase*.
  - Oncovirinae subfamily- oncogenic: cause leukemias, lymphomas, mammary and neuronal tumors.
    - Human T cell leukemia virus (HTLV1 & 2)- T cell leukemias
    - Human immunodeficiency virus (HIV 1 & 2)- AIDS

Lytic vs Lysogenic Viruses

- Lytic viruses- When they infect the cell the ultimate fate of the cell is death.
- Lysogenic- Lysogenic viruses have an intermediate stage in which they infect a host cell and then usually integrate into host cell DNA (*prophage* or *temperate phage*).
  - Virus must be a DNA virus or have a DNA intermediate stage
  - Cannot find infectious particles in cell shortly after infection
  - May stay dormant for years or forever (need appropriate stimuli)

Lysogenic Integration

- Lambda Phage (λ)- linear genome that must circularize prior to integration, which is followed by a reordering of the genes after insertion. λ phage DNA has "sticky ends" which provide the mechanism to become circularized once in the infected cell. Location of integration is specific in this instance (specialized *vs* generalized transduction)
- In order to integrate viruses need specific enzymes, such as *int* (integrase protein) and ligases.

Excision of Integrated Genome

- Excision- some integrated genomes must be excised prior to replication, others cause replication of viruses while still integrated. Errors can occur in the excision process and in the replication process in copying NA while still integrated. Resulting phage is defective in replication
- Superinfecting phage DNA does not replicate in a lysogen. There is some sort of "immunity substance" synthesized by the prophage which prevents expression of most of the prophage genes as well as inhibiting expression of identical phage genes that infect in other sites. Immunity only specific for same phage.

Benefits of Lysogeny

- Provides mode of persistence which does not deplete the supply of hosts for the obligate parasite.
- Opportunity for extended growth under non-selective conditions in which multiple genetic variations can occur
- Temperate phages can confer new characteristics to host (lysogenic conversion) (*Corynebacterium diphtheriae* toxin production)

Viral-Host Cell Interactions

- Lytic, persistent, latent, transforming and abortive
  - (1) a prerequisite for any of these types of infections is the initial interaction between virus and cell receptor (if cell lacks receptor there is no infection and (2) that both virus and cell play role in interaction, i.e., same virus in one cell type will be lytic and in another cell type will be latent.

Lytic Infections

- Virus inhibits DNA and RNA synthesis and protein synthesis of host cell
  - **Persistent** infections: result in continuous production of infectious particles. Generally, cell death is balanced by new cells produced by division with no net loss (antibody/IFN help, etc…)
  - **Latent** infections: existing, but not exhibited. Some virus specific proteins are synthesized, but infectious virus is not formed. (herpes viruses and some tumor viruses)
  - **Transforming** infections: DNA viruses and RNA tumor viruses- alter cell morphology, and characteristics) Integration necessary.
  - **Abortive** infections: Non-permissive cells or non-infectious particles (# of progeny decreased or specific protease not available to cleave certain proteins).

How do viruses kill cells?

- Cytopathic Effects (CPE)- some viruses do not kill cells in which they multiply
  - inhibition of host metabolism (transcription and translation)
    - effect on Na^+/K^+ pump may allow for viral transcription but not host cell transcription
    - so much viral mRNA that it out competes host mRNA for ribosome sites
    - trigger apoptosis
    - reduction of plasma membrane

The Immune System

- Innate vs Adaptive Response
  - non-specific
    - skin, mucous, acid, macrophages, NK cells, neutrophils, tears, IFN, other cytokines (IL-2)
  - specific
    - T cells (cellular)-- cellular cytotoxicity
      - cell bound antigens (virally infected cells, cancer cells, tissue transplant cells)
    - B lymphocytes (humoral)-- antibody responses
      - soluble antigens (bacteria, toxins, free viruses)

T Cell Responses

- Must see antigen in association with "self MHC" along with "foreign antigen". [T cell receptor]
  - antigen is processed by antigen presenting cells (APC)
    - macrophages, dendritic cells, langerhans cells, B cells (those with MHC class II molecules)
    - cytokine synthesis and release
    - activates other T cells (T helper {$CD4^+$, T_H}, T cytotoxic {$CD8^+$, T_C}), as well as activating B cells
- Educated in thymus- only those cells that recognize self MHC and foreign antigen are kept, others killed

B Lymphocyte Responses

- Humoral response-- learn self from non-self in bone marrow, when activated the plasma cells produce antibody
- One B cell makes one specific antibody, but can change antibody class (class switch)
  - Five types of antibodies--
    - IgG- 2º Ab, crosses placenta, activates complement (C')
    - IgA- dimer, crosses epithelial cells
    - IgM- 1º Ab, pentameric, activates C'
    - IgE- allergic antibody type, binds to FcR of mast cells
    - IgD- found only on immature and mature cells prior to the cell being activated by antigen. Once Ag stimulated it disappears

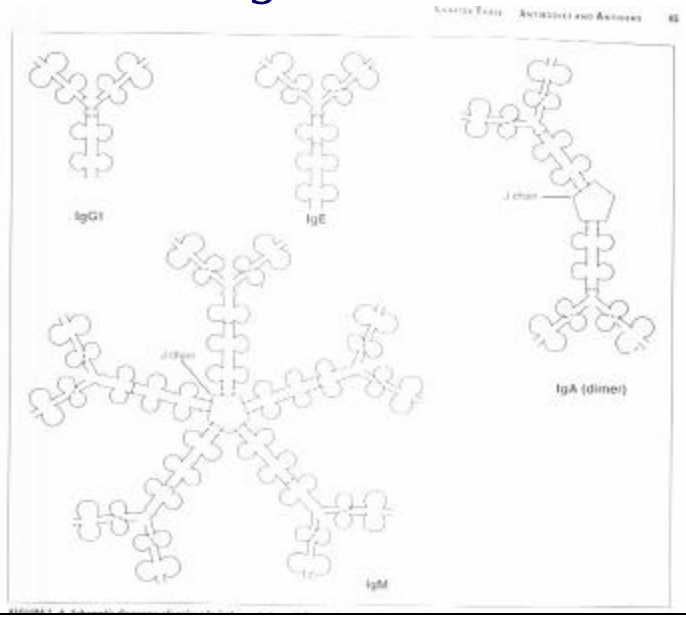
Antibody Action

- *Opsonization*- "greased pig effect" enhanced phagocytosis
- *Complement Activation*- membrane lysis: oly activated when Ab bound to specific Ag (Ab:Ag complex)
- *Blocking*- bind to and block receptors
- *Neutralization*- inhibit action of enzymes, toxins, proteins

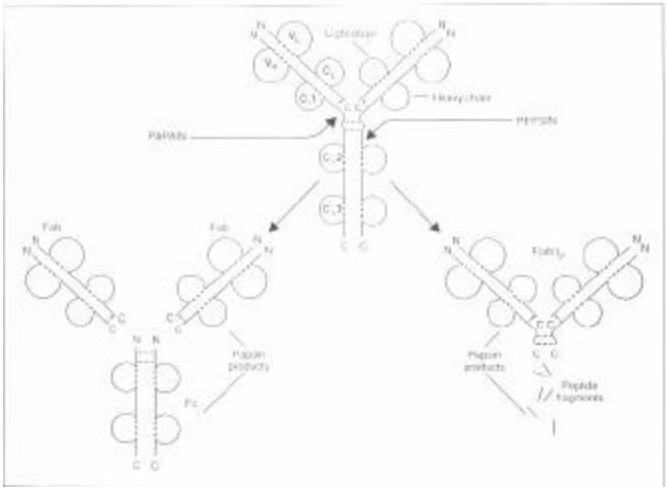
Antibody Structure

- Composed of 2 Light and 2 Heavy peptides
- Composed of Variable and Constant regions
  - variable is where Ag binds and its specificity is due to interactions between the Light and Heavy chains
- Has Fab, $F(ab)'_2$, and Fc Regions
  - Fab- fraction antigen binding
  - Fc- fraction crystalizable (binds C', and receptors on mast cells and macrophages), gives specific properties to Ig molecules (crossing placenta, etc…)
- Each segment comes from different genes (V,D,J,C)

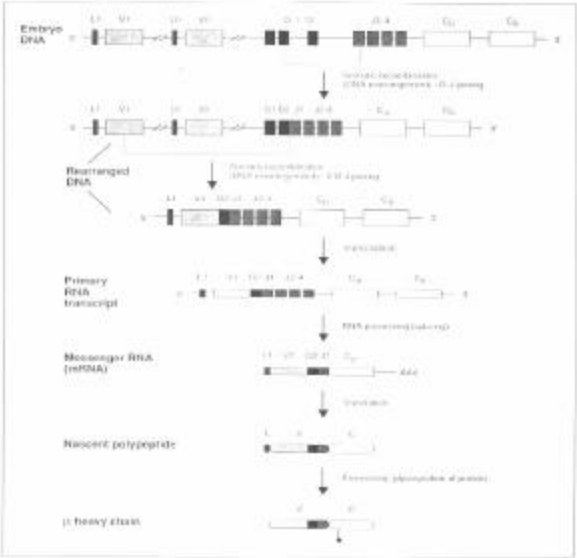
Immunoglobulin Structure



Ig Fragments



Ig Genes



Interferons (IFN)

- 1957- Alick Isaacs & Jean Lindenmann: incubated chorio-allantoic membrane from embryonated chicken eggs with heat-killed influenza virus, washed, incubated another 24 hours and checked buffer for anti-viral activity. Did this by placing a fresh membrane in this buffer and inoculating with infectious influenza virus. Found virus did not grow. Substance called *interferon*.
- 3 types of IFN: α β γ The α (leukocyte IFN- 20 different families coded for by different genes) and β (Fibroblast IFN) are similar and are synthesized by most cells. The gamma is released by stimulated T cells by Ag for which they are specific.

Interferon (IFN)

- IFN
  - enhances the activation of NK cells
  - inhibits cell division of tumor cells [anti-cancer effects??) (& normal cells)
  - enhances macrophage activity (MAF)
  - up regulate expression of MHC I and gamma increases MHC I and II (viruses decrease levels of MHC on infected cells)

Mechanism of Action of IFN

- Induction- results in **(1)** de-repression of IFN genes, the release of IFN protein and **(2)** results in an antiviral state in other cells by the release of IFN
- Induction of human IFN is controlled by chromosome # 9, with the IFN receptor gene on chromosome # 21
- Believed that dsRNA is specific inducer (even DNA viruses must go through some intermediate step in which dsRNA is made)

Interferon gamma

- Is not stable at pH2 as alpha and beta IFN's are
- Is different in its sequence (alpha and beta much more similar to each other than to gamma)
- Is not stimualted by dsRNA but by activation of T cells by specific antigen
- Stimulates immune response in different ways than alpha or beta

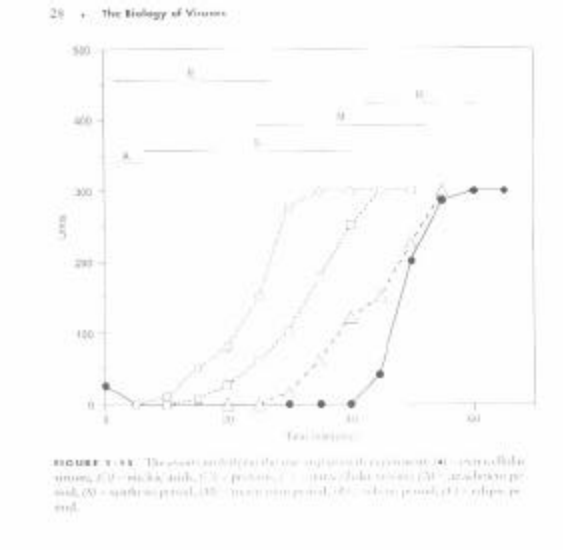
Mechanism of Action of IFN

- **dsRNA (synthesized by infecting virus) stimulates phosphorylation of cellular proteins and causes synthesis of certain proteins:**
  - Phosphorylates initiation factor E1F2ά- impairs initiation of protein synthesis in host
  - activates 2’5’-A synthetase (inactive without dsRNA), which synthesizes 2’5’-A which activates RNaseL
  - activates RNaseL, a ribonuclease, which cleaves mRNA to stop protein synthesis
- **When IFN released it binds to IFN-R (made when stimulated by dsRNA) and induces anti-viral state in cell**

The Process of Infection

- 6 Stages of Infection
  - attachment (adsorbtion)- receptor-to-receptor
  - Penetration- movement through membrane
  - Uncoating- release of nucleic acid
  - Synthesis- replication of viral NA, translation
  - Replication- putting together of nucleocapsid
  - Release- movement out of viral progeny

One-Step Growth Curve



The Process of Infection

- Attachment- due to diffusion and attachment of viral receptor to cellular receptor. The cell receptor usually has other functions to support the cell in normal functions
  - most phages attach to cell wall, but may also attach to pili, flagella or capsule
  - in animal cells the receptors are highly specific for a particular virus
  - viruses can sometimes use different molecules as receptors

The Process of Infection

- Penetration (Hershey & Chase)- only NA enters cell
  - in phage, the tail is contractile. Following attachment the tail core is pushed through when contractile rings are compressed and DNA injected into host cell. Action aided by lysozyme and 144 molecules of ATP
  - other phages AND animal viruses have both the coat protein and NA enter the cell
  - Enveloped viruses enter cell by fusing their membrane with host cell membrane/ others enter by **receptor-mediated endocytosis**

The Process of Infection

- Uncoating- once the virus nucleocapsid is entered into host cell the NA must be released. Uncoating is dependent upon a decrease in pH of the vesicle to pH5-6. This causes a conformational change in capsid proteins and allows NA to release
  - the nucleic acid is not "naked" but remains associated with internal structural proteins (includes enzymes necessary for NA synthesis or helps NA associate with ribosomes
  - Most NA seems to be degraded (inefficient)

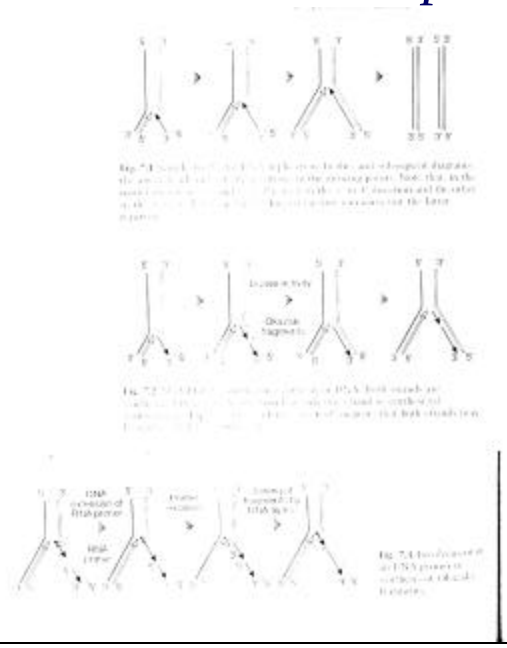
Prevention of early stages of Infection

- Block cell and/or viral receptors-- if you do this it may prevent normal function of host cell, or prevent attachment and penetration of virus into cell
- Amantadine-- used to prevent influenza virus infection by elevating the internal pH of endosomes thereby preventing the uncoating of the virus
- For plants vectors should be eliminated

Replication of viral DNA

- Semi-conservative replication= each daughter molecule contains one parent strand and one new strand.
  - DNA polymerase- reads parent strand in 3'-->5' direction and continuous new strand is made in the 5' to 3' direction. Discontinuous synthesis occurs in the 3' to 5' direction (Okazaki fragments) after the parent strands have opened up
  - DNA polymerases cannot start strand *de novo*, but require RNA polymerase to start short primer

Semi-Conservative Replication



Viral DNA Replication

- Okazaki fragments- require short RNA primer for each fragment and each fragment is then ligated after RNA primer is removed.
  - Rifampicin- inhibits RNA polymerase. Can have rifampicin resistant RNA polymerases.
- In DNA replication there is only one mistake made in 10^9-10^{10} base-pair replications compared to 10^3-10^4 errors in RNA genomes. DNA polymerases proof read what they synthesize.

Viral DNA Replication

- DNA polymerases have ability to synthesize in the 5'--->3' direction, have the ability to proof-read, and have exonuclease activity to remove 3' bases if in error
- When the RNA primer is removed there is a gap that requires 3'-->5' synthesis to fill. Since this cannot occur the problem is resolved by ***concatemer*** formation. If this did not occur the strands would get shorter and shorter.

Viral DNA Replication: Concatemers

- Sticky ends enable concatemers to be produced, so that the shorter 5' ends of the daughter strands would connect to sticky ends of other complementary daughter strands. Formation of unit-length molecules would occur by the action of endonucleases making specific breaks in the concatemer chains

Replication of Circular DNA

- Replication of ssDNA always involves a ds intermediate
  - 30 minutes after infection with λ (dsDNA) phage the DNA from infected cells can be separated into 3 classes: lenear monomers, covalently closed circles, and concatamers
  - most of the label is found in concatamers and can be chased into monomers (pulse/chase experiments)
  - concatamers can be generated by rolling circle model
  - monomer linear-->circle-->concatamer-->monomer

Circular Genome Replication

- Replication in bidirectional manner for circular DNA's
- For ssDNA (ϕX174) the infecting single strand is converted to a ds replicative intermediate (RI) form. The leading (continuous) strand is synthesized on the complementary strand, and the Okazaki fragments are synthesized on the viral strand.

Replication of linear ssDNA

- Parvoviruses-- there is a ds replicative (RI) intermediate form of the DNA
- If the virus has modified bases, the viral genome must first synthesize enzymes that are specific for replication of viral DNA containing these bases, and these enzymes are found in early synthesis.

RNA Synthesis

- Synthesis of RNA involves
  - replication- production of progeny virus genomes
  - transcription- production of RNA complementary to the genome (from + or - strands, not necessarily mRNA)
  - No DNA intermediate involved (except for retroviruses)
  - dsRNA isolated (RNase-resistant)- called *replicative form* (RF)
  - there is also a dsRNA with single stranded tails (RNase sensitive)= *replicative intermediate* (RI)

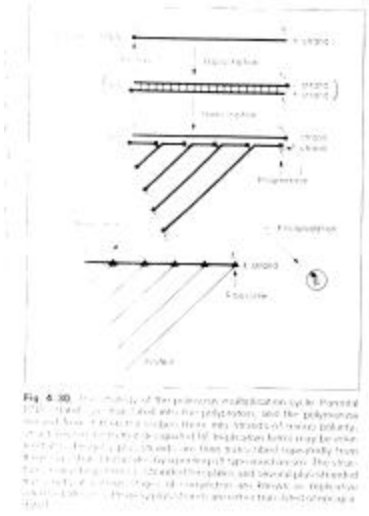
RNA Synthesis

- Roles of RF and RI in the replication of viral RNA is not known
  - RF results from annealing of free + and minus strands. During synthesis, the region of hydrogen bonding is confined to the sequences covered by the replicase , and the enzyme makes and breaks H-bonds as it proceeds (i.e., there are short ds sequences as RNA is replicated, and there may be many sites of these with ss strands of RNA coming off because of numerous initiation sites.)
  - minus strands are then copied into plus strands
  - 10X more plus strands made than minus because of packaging of plus strands (no negative feedback) and - strands stay to act as template

RF and RI Intermediates: Poliovirus

RF

RI



+ strand

- strand
+ strand

+ strand & protein syn

RNA Synthesis: Class V viruses (ss, -polarity)

- Requires a pre-existing RNA-dependent RNA polymerase to make mRNA from - strand
- viral RNA synthesis is not affect by either actinomycin D or alpha-amanitin (specifically inactive eucaryotic RNA polymerases which make mRNA)
  - Influenza virus is unique: viral mRNA synthesis requires the synthesis of cellular mRNA and all viral RNA synthesis takes place in the host cell nucleus.
    - Viral transcriptase complex contains 3 proteins: PB1, PB2 and PA. PB2 recognizes the 5'-methyl cap of cmRNA and syn 10-13 nucleotides, PB1 elongates this primer with viral mRNA, and PA replicates - stranded RNA.

RNA Synthesis of Class III Viruses (ds RNA, Fragmented)

- Reoviruses-- dsRNA replicated *conservatively*
  - double capsid, contains RNA polymerase activity
  - if pre-label infectious viral cores with 3H-uradine, all labeled RNA parental strands stay together while progeny virus is unlabeled.
  - All + strands made off of -RNA strand of dsRNA and act as mRNA as well as being encapsidated as ss RNA with replicase activity which then makes - strands .
- Packaging of 10 segments occurs thru random assortment of segments in a common pool before the + strand enters the - strand synthesizing particle

DI Particles

- DI= defective interfering particles
  - all RNA and DNA viruses produce DI particles as the result of errors in NA synthesis. These are deletion mutants which are unable to reproduce themselves without the assistance of the infectious parental virus.
  - DI genomes actually interfere with the yield of infectious progeny by competition for limited amount of some product synthesized only by parent virus.
- RNA satellite viruses= plants- cannot multiply without a helper virus (specific). Not DI viruses!

RNA Viruses with DNA Intermediate

- Retroviruses- RNA tumor viruses
  - RNA-dependent DNA polymerase (reverse transcriptase) carried with virus
  - each virion contains two copies of identical RNA which are linked together (these have + (mRNA) polarity, poly-A tail, and 5' cap.
  - Genome contains 3 primary genes--
    - *gag*- encodes 3 group specific antigens (virion proteins)
    - *pol*- encodes 3 minor virion proteins and **RT**
    - *env*- encodes virion envelope proteins

Retrovirus Replication

- ssRNA converted to linear dsDNA in cytoplasm. This migrates to nucleus and integrates into cell DNA.
- Integration requires the integrase enzyme coded for the the *pol* gene. Enzyme must enter the cell with the particle to be most effective
- Integration is NOT required for production of progeny virus!

Properties of Reverse Transcriptase

- RT requires a primer to initiate RNA-->DNA transcription. Can be as short as 4 bases, and is a cellular tRNA (each different oncovirus contains only one type of tRNA).
- RT composed of 2 subunits:
  - an α-subunit has enzymatic activity
    - synthesizes DNA from RNA
    - synthesizes DNA from DNA
    - digests the RNA strand from RNA:DNA hybrids (ribonuclease H)
  - a β- subunit that binds to the tRNA primer

Integration

- 3 steps-
  - 1. Two bases are removed by viral integrase from 3' end of linear vDNA.
  - 2. The 3' ends are annealed to sites a few bases apart in the host genome, which is then cleaved
  - 3. Gaps and any mismatched bases are repaired
- Cells contain 1-20 copies of integrated proviral DNA. There are no specific sites of integration
- From integrated DNA new viral RNA genomes are synthesized by cellular RNA polymerases.

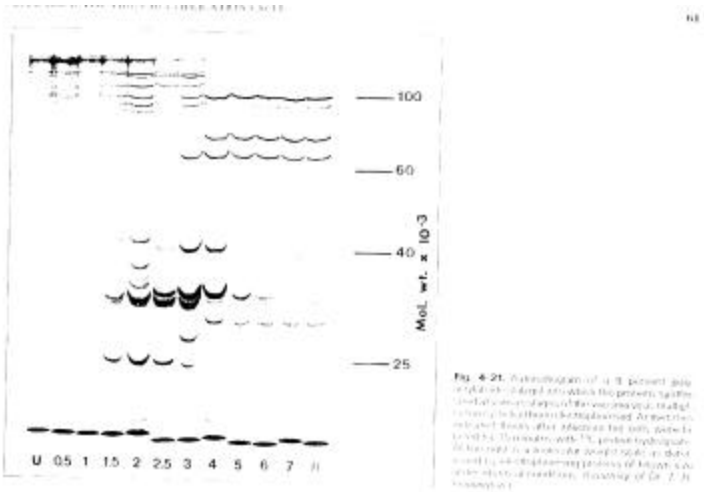
Hepadnaviruses

- HBV infects 2 X 10^8 people world wide, causing acute liver disease and is the single most important cause of viral induced cancer in humans.
- Genome composed of linear DNA that has - strand covalently linked to a protein at its 5' end, and a complementary + strand, which is an incomplete copy. The + strand overlaps the 5'--3' junction of -DNA strand and enables the - DNA strand to circularize.
- Virus carries own polymerase
- Virus has envelope that contains surface (S) antigen. Ab provides protection
- Has a RNA intermediate (replication blocked by actinomycin D which blocks DNA directed RNA syn). RNA necessary to make + DNA strand.

Regulation of gene expression

- When a virus infects a cell not all genes are expressed at the same time: Early & Late genes
- Discovered through pulse-chase experiments
- For DNA viruses regulation can be at the transcriptional or translational levels:
  - change in initiation specificity of RNA poly
  - de novo synthesis of new RNA poly with diff spec
  - synthesis of positive or negative control proteins

Autoradiogram- time of protein synthesis



Regulation of gene expression

- Genomes of small RNA bacteriophages can code for only 3 proteins: phage coat protein, maturation protein and RNA synthetase. Proteins expressed in the ratio of 20 coat: 5 synthetase: 1 maturation (same RNA strand is transcribed at different rates for each gene)
  - there are initiating regions at each gene which bind ribosomes directly (with diff affinity?), with different frequencies of initiation rates (due to conformation of viral RNA)

Regulation of gene expression

- RNA of picornavirus in translated as a large poly-peptide that is cleaved post-translationally. All cleavages carried out by viral specific proteases

- The class V viruses have segments and each segment codes for one monocistronic mRNA (one mRNA makes one protein). Influenza A has 8 segments (8 proteins) not in equal amounts.

Regulation of gene expression

- Orthomyxoviruses-- unique among "true" RNA viruses in that they have an essential part of their replication cycle within the nucleus.
  - Uncoated particle is transported to nucleus where viral RNA is synthesized, and later in infection the proteins that are made in the cytoplasm go into the nucleus (control very tight as to what proteins can pass through the nuclear membrane [also true of DNA viruses])
  - HA protein must undergo proteolytic cleavage before it is functional

Regulation of gene expression

- All DNA viruses (with exception of pox viruses) synthesize their mRNA from dsDNA in the nucleus. Largest viruses can synthesize when host cell is not in S phase, but smaller viruses cannot.
- Viruses of intermediate size can induce host cell to undergo division.
- With the exception of pox and parvo-viruses all DNA viruses can transform host animal cells.

Regulation of gene expression

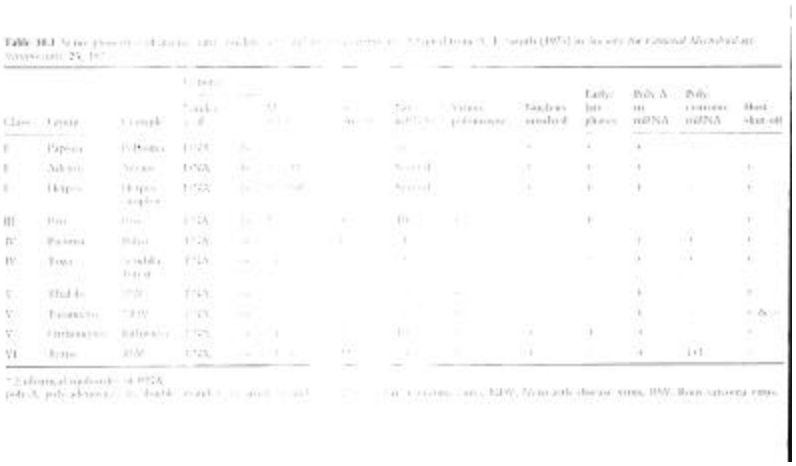
- Papovaviruses-- (SV40)
  - DNA transcribed by cellular RNA polymerase
  - major early protein (T [tumor specific] antigen) and a lesser small t. Come from same coding area
  - RNA's formed by splicing of exon regions after introns not transcribed.
  - Large T and small T mRNA's are about same size, but only initial part of small t mRNA translated because of stop codon (UAA). The mRNA of large T has a splice taken out the has stop codon in it.

Regulation of gene expression

- T antigens of polyoma virus are similar. The start for all 3 T antigens is identical but there are three different introns that are removed so that all three reading frames can be read and produce 3 proteins
- Early phase expression of proteins is followed by inductin of the synthesis of host cell enzymes and of host cell DNA. Large T antigen binds to viral DNA and allows replication to proceed.

Regulation of gene expression

- Early and late mRNA's transcribed from different strands. Large T antigen down regulates the transcription of early genes and up regulates late gene transcription (virion proteins)



Adenovirus/Herpes & Pox Virus Replication

- Adenovirus-- Linear genome-- early and late synthesis carried out by cell polymerase on both strands. Early transcription (pre-DNA synthesis) takes place from 5 regions and each has its own promoter and termination sequences. Late synthesis occurs only after DNA synthesis
- Herpes-- Linear genome- only difference lies in late synthesis occurs independent of DNA synthesis
- Pox-- Linear genome- only DNA virus to replicate in cytoplasm. Has own DNA-dependent RNA polymerase as well as other enzymes (capping, methylation, poly-A)

Retrovirus Replication

- DNA provirus is synthesized in the cytoplasm and transported to the nucleus where it is integrated into the host genome.
- 3 mRNA's synthesized (*gag*, *pol* & *env*). Usually only gag is made unless there is suppression of the non-sense codon. Gag is processed by a protease to produce 3 major internal virion protens (matrix, core and nucleo-capsid). Gag-pol polyprotein processed to produce protease, RT and integrase that go into core.

Viral Assembly

- 3 ways in which virus-specific proteins and NA's come together:
  - self-assembly- spontaneous combination
  - genome may specify certain morphogenetic factors which are not structurally part of virus but are required for assembly
  - may assemble from precursor proteins which are modified to form virion, and these are not possible to dissociate and then reassemble into mature particle.

Viral Assembly: Self-Assembly

- Absolute proof of this requires that the purified viral nucleic acid and purified structural proteins be able to combine *in vitro* to give particles that resemble original virus in size, shape and infectivity. The virus must then be disassembled and the released subunits reassemble in a specific manner (proper assembly is the test for proper disassembly).
- TMV- can be assembled from components and capsid can be assembled without RNA component.

T4 Viral Assembly

- Tailed bacteriophage-- studies come from non-permissive E. coli infected with phage containing amber mutations in structural genes. When examined cannot see phage particles, but can identify structures in cell that are phage components. Cells with mutations in genes 34, 35, 36, 37 or 38 accumulate phage particles which appear normal except for the absence of tail fibers. These genes codes for tail fiber proteins. Using this technique the researcher can find the function of most of the viral genes. This technique can now be done *in vitro*.

T4 Viral Assembly

- Base plates, heads and tails each have their own pathway. As completed products are made they spontaneously come together and then certain proteins are modified to secure them to each other.
- There are "scaffolding proteins" which help in the assembly of mature particles but are not found in the mature virion. These can be re-used.

Assembly of enveloped Viruses

- Herpes viruses-- replicate in the cell nucleus, but viral proteins synthesized in the cytoplasm and transported to nucleus. Virus assembled in nucleus and as it passes thru nucleus picks up nuclear membrane. Prior to budding the membrane is modified by the addition of viral specific proteins and these proteins are glycosylated.

Assembly of enveloped Viruses

- Majority of enveloped viruses get envelope by budding through plasma membrane or one of the internal membrane structures.

Virus--Host Interactions

- 7 classifications, but a single virus may reach across spectrum of these divisions:
  - Acute infections
  - inapparent infections
  - chronic infections
  - persistent infections
  - latent infections
  - slowly progressive infections
  - tumorigenic infections

Virus--Host Interactions

- Acute Infections
  - analogous to one-step growth curve-- based on symptoms and laboratory tests (w/o this no ID can be made). Lab tests (isolation and titration, detection of viral antigens in blood or tissues, PCR, EM).
  - In acute infections virus replicates and cell dies
  - Most people recover within 3 weeks: IFN, Ig, Tc cells, NK cells

Virus--Host Interactions

- Inapparent Infections: no signs or symptoms of disease. Lab tests confirm infections. Virus has evolved a favorable equilibrium with host (polio virus causes no symptoms in 90% of infections). These infections have same duration and are cleared by same mechanisms as acute infections.

Virus--Host Interactions

- Chronic/Persistent Infections-- immune system cannot get rid of virus
  - in a chronic infection there is a large amount of virus or viral Ag produced. Immune system may be inhibited or MHC molecules may be reduced or IFN production may be reduced (page 218, table 15.3)
  - persistent infection does not have as large a viral load

Virus--Host Interactions

- Latent Infections: no infectious virus is present in animals with latent infections. Latency maintained by a balance between virus and immune system. Breakdown of this balance leads to acute infection.

Virus--Host Interactions

- Slowly progressive infections-- two types: slowly progressive diseases caused by viruses and spongiform encephalopathies caused by unknown agent
  - slowly progressive- measles infection: in small % virus establishes itself in brain and 2-6 years later causes degenerative changes in brain function= subacute sclerosing panecephalitis (SSPE) (due to infection early [<2 yrs]). Infectious virus cannot be isolated, but cultured cells can be infected by brain extracts. These viruses are mutated from original.

Virus--Host Interactions

- Spongiform encephalopathies-
  - Scrapie virus of sheep- causes animal to scrape itself against things. Cannot isolate infectious agent, but brain suspensions can transfer infectin to susceptible animals.
  - May be a **Prion-** infectious agent does not have NA.
  - Commonly called Mad Cow Disease
- Creutzfeldt-Jakob- slowly progressive disease of CNS in humans. Agent not been isolated, but can be transmitted (surgical instruments) (1:million/year)

Virus--Host Interactions

- Virus-induced tumors-
  - either caused by DNA viruses or retroviruses. DNA viruses mostly cause acute infections and rarely cause tumors
    - Epstein-Barr virus causes mononucleosis, but in this country does not usually cause Burkitt's lymphoma. (Malaria may be predisposing factor)
    - Cancer needs more than one mutation, and virus may transform cell but cell will not cause tumor in animal.
    - Insertion into gene/growth factor/oncogene

Virus--Host Interactions

- How virus is transmitted from infected individual to susceptible host:
  - respiratory-middle sized droplets (0.4-9 um size)
  - fecal-oral route- polio
  - urinogenital tract- HBV, AIDS, HSV, papilloma
  - mechanical
- vertical transmission- mother --> fetus
- horizontal transmission- between individuals

Virus--Host Interactions

- Mechanical transmission
  - breaks in skin, biting arthropods
    - mosquitoes (AIDS?), ticks, mites, fleas
    - contaminated needles
    - fomites?? (viruses do not survive long outside body)

Genetics of Animal Viruses

- Types of virus mutants
  - Spontaneous mutations constantly occur during multiplication. Many mutations are lethal, but others are not.
  - RNA and DNA viruses can be mutated by nitrous acid, or hydroxylamine (DNA viruses by UV). These create random mutations.
  - Produce mutations by point mutation or by deletion mutation (frameshift mutations)
  - Point mutations are called **conditional lethal mutants**

Genetics of Animal Viruses

- Conditional Lethal Mutants:
  - can multiply under some, but not other conditions
    - temperature sensitive mutants- amino acid substitution will not allow protein to maintain shape at certain temperatures, but will at permissive temperatures.
    - Host-dependent mutants- codon for some AA is changed to a termination codon (UAG, UAA, UGA). Some mutant bacterial strains have mutated tRNA that recognize these termination codons and insert an AA in this place and allow production of full length protein. Maybe protein can still function? The mutations that give rise to UAG, UAA and UGA are known as **amber, ochre & opal** respectively

Genetics of Animal Viruses

- Under conditions of multiple infection, cells may become infected with 2 or more virus particles with different genomes. If they are closely related (belong to same genus) they can interact genetically.
  - Recombination- viral genomes that consist of ssRNA do not recombine; need fragmented RNA. This is the cause of antigenic shifts that have occurred with influenza viruses. (13 types of H and 9 types of N)

Antiviral Chemotherapy, IFN & Vaccines

- Must find where in virus multiplication cycle that you can best interrupt with chemicals
- Must make sure that host cell is not affected
- Chemotherapy & IFN meant to control viral disease by arresting and curing infections once they have started
- Vaccines prevent viral infections and prevent the onset of disease

Target cell approach

- Should use drugs that could only enter or be activated in virally infected cells:
  - one of early herpes virus specific enzymes produced is a deoxypyrimidine kinase able to phosphorylate compounds that cannot be phosphorylated by any cellular enzyme. Can use deoxyriboside analogues that are phosphorylated, and activated, only in cells that are infected, thereby stopping DNA replication
  - infected cells have increase membrane permeability and therefore certain compounds taken up by these cells where normal cells do not.
  - Coupling inhibitors to monoclonal antibodies

Antiviral Chemotherapy, IFN & Vaccines

- **Antiviral therapy by chemotherapy:**
  - **attack adsorption,penetration & uncoating-- not attempted seriously because not enough is known about specific individual reactions. Alpha-adamantanamine does inhibit uncoating of influenza viruses but mechanism unknown.**
  - **Replication of NA's-- synthesis of many viruses catalyzed by enzymes not found in normal cells (all RNA viruses and Pox viruses). Should be possible to isolate and identify these.**

Antiviral Chemotherapy, IFN & Vaccines

- **Integration of viral genomes into cellular genomes-- Some viruses use host enzymes for this purpose but others use viral specific enzymes. If latter is true then can attack these enzymes as targets for chemotherapy.**
- **Synthesis of viral mRNA's-- synthesis coded for by virus specific enzymes (polymerases). Post transcriptional modifications also by virus specific enzymes. Use these as targets.**
- **Synthesis of viral proteins-- viral mRNA differs in some way from host cell mRNA. This difference should be exploitable.**

Antiviral Chemotherapy, IFN & Vaccines

- **Viral morphogenesis-- many viral capsid proteins are synthesized in the form of precursors that are cleaved to furnish the actual proteins used for the formation of virus particles. The enzymes that cleave these precursors are highly specific. It should be possible to characterize them and design inhibitors for them (i.e., protease inhibitors for HIV infections).**

Antiviral Chemotherapy, IFN & Vaccines

- **Many compounds inhibit virus multiplication very effectively in cultured cells and have no adverse effects on the growth of uninfected cells, but most of them are very inefficient at killing viruses *in vivo* and/or they have adverse side-effects. The compounds that do work:**
  - **Isatin-beta-thiosemicarbazone (IBT)-- potent inhibitor of pox virus multiplication. Viral DNA replication and transcription occur normally, but translation of late mRNA is inhibited (no capsid proteins)**

Antiviral Chemotherapy, IFN & Vaccines

- 2-hydroxybenzylbenzimidazole (HBB) and Guanidine: inhibit multiplication of many picornaviruses. They interfere with replication of viral RNA (prevent the synthesis of of progeny plus strands). Both drug resistant and drug-dependent viruses emerge.

- Rifampin-- bind to bacterial RNA polymerases and prevent the initiation of transcription. Does not bind to animal RNA polymerases, but does bind to pox and adenoviruses. Involves some event in viral morphogenesis.

Antiviral Chemotherapy, IFN & Vaccines

- Arildone, Rhodanine, and WIN 51711-- inhibit the uncoating of poliovirus. Do not affect adsorption or penetration, but prevent the pH dependent uncoating in acidic endosomes. They somehow increase the stability of the virus (4 hours after infection infectious virus can be recovered).

- Alpha-adamantanamine-- inhibits influenza virus by inhibiting the fusion of the viral and endosomal vesicle membranes so nucleocapsids cannot be released. Prophylactic effect for influenza A. FDA approved.

Antiviral Chemotherapy, IFN & Vaccines

- Analogues of Ribonucleosides and Deoxyribonucleosides
- **recognized as nucleic acid building blocks by polymerases, and once incorporated into NA they interfere with NA functioning properly.**
  - **Example: 5'-iodouracil- (IDU) analogue of thymine and is incorporated into DNA. Does not base-pair with adenine and mismatching occurs during replication and transcription.**
  - **Analogues may interfere with enzymes in pathways that lead to RNA or DNA synthesis (anti-cancer agents). Any enzymes specifically encoded for by viruses will be affected to greater extent**

Antiviral Chemotherapy, IFN & Vaccines

- Adenosine arabinoside (Vidarabine, Ara-A)--
  - antiherpes drug- Good *in vitro* activity. Inhibit herpesvirus DNA polymerase more than cellular DNA polymerases and act as chain terminators. They are rapidly deaminated and lose potency. Pill and topical forms.
- Deoxyribonucleoside analogues--
  - derivatives of thymine or cytosine in which the methyl group is substituted by arabinose. **ACYCLOVIR:** terminates DNA synthesis. No activity against latent infections

Vaccines

- Only means of preventing diseases caused by viruses is through activation of the immune system. Three types of antiviral vaccines: inactivated virus, attenuated active virus, and subunit vaccines.
- Vaccines should have following properties:
  - cause less severe disease than the natural infection
  - stimulate effective and long-lasting immunity- correct Ig in correct place
  - be genetically stable- must not revert to virulence

Vaccines

- **<u>Inactive vaccine</u>-** must be sure it is dead without loss of antigenicity. Not easy to do both. Must kill all infectious particles (not all inactivated at same speed). Use of whole virus!
  - Do no multiply and cannot get to site **where virus would normally be found**
    - UV- can still express genes that are not hit
    - Photodynamic (dyes that intercalate between nucleic acid base pairs), but genes that have not been hit remain active
    - formaldehyde- destroys infectivity by reacting with amino groups of adenine, G & C. ssNA's are readily inactivated but dsNA's are not. Cross-links proteins
  - Salk vaccine for polio (1953)
  - Can immunize with no more than 3-4 antigens at a time (MMR)

Vaccines

- Attenuated Vaccines-
  - Most common method for attainment of attenuated strains is by repeated passage of the human pathogen in other host species, which results in the select6ion of multistep variants with drastically reduced virulence for humans
    - effective in very small amounts because they can multiply. Viral progeny act as immunogen, not innoculum.
    - Stimulate correct type of antibody, and at correct site
  - Types of attenuated viruses
    - yellow fever
    - polio strains 1, 2 & 3 (Sabin)
    - mumps, measles, rubella
    - rabies

Attenuated Virus Vaccines

- When would you use killed vaccine over attenuated vaccines?
- **in young children, who might develop a more sever infection than adults**
- **must give after weaning (maternal antibodies might block response). Give 2-6 months after weaning.**
- **Certain clinical conditions predisposed people to more severe disease**
- **fetal development might be affected and cause birth defects**
- **prior virus infection might interfere with the multiplication of a live vaccine and immunity not established.**

Vaccines

- Too late to immunize once symptoms appear
  - **Passive Immunity**-- last only as long as Ig lasts in body. Pre-formed Ig's from other source: no active response from your own body.
  - What sources??
    - Human- pooled serum (problems with contamination)
    - animal (problems with allergies)
      - horses
      - rabbits

Vaccines

- SubUnit Vaccines-
  - most of particle is not imp't in resistance to disease. Can pick specific component and make Ab's to it.
  - Problem might be a change in shape vs intact Ag.
  - Problem might be that virus mutates Ag.
  - Problem in determining importance of Ag in blocking infectivity
- If virus cannot be grown in culture (HBV), technology allows for cloning of Ag and expression in yeast or other vector

Carcinogenesis & Tumor Viruses

- 1908: Ellerman & Bang demonstrated that spontaneous leukemias of chickens could be transmitted to other chickens by cell-free filtrate.
- 1911: Rous found that a chicken sarcoma can be similarly transmitted
- These viruses are retroviruses
- 1936: Bittner demonstrated that a spontaneously occurring mouse adenocarcinoma is caused by a virus transmitted from the mother to the progeny through milk.

Carcinogenesis & Tumor Viruses

- Cancer in rodents caused by :
  - polyoma virus
  - simian virus 40 (SV40)
- Cancer in humans caused by:
  - adenovirus
  - papilloma
  - herpes
- When a cell is transformed, **the virus that has induced the cancer in generally no longer recognizable in culture (by infectivity) or by antigenicity. Can ID genome (PCR).**

Carcinogenesis & Tumor Viruses

- Two theories of oncogenesis:
  - deletion of genetic material from cell which is essential for normal functioning
  - activation of specific genes which could have positive or negative control over cell growth
- Formation of cancer involves at least two mutations in cell
- Virus can induce cancer by
  - part of viral genome expresses a gene (oncogene) which initiates and maintains transformation
  - alters control of expression of cellular genes
  - causes immunosuppression

Carcinogenesis & Tumor Viruses

- Oncogenes of DNA viruses:
  - Papovavirus- Polyoma virus (agent of many tumors): produces various kinds of neoplasia when injected into newborn mice. Similar in structure to SV40. Human papovaviruses isolated from brain of patient with progressive multifocal leukoencephalopathy (PML).
  - 70% of people have antibodies to papova viruses

Transformed cells

- Properties
  - DNA is either integrated into host genome or is present as a plasmid in the nucleus
  - cell is immortal, loss of contact inhibition, growth in soft agar
  - transformed cells contain either the entire viral genome or a fragment of the genome
  - transformed cells give rise to tumors when injected into susceptible host
  - differ in morphology: more rounded, more mitotic figures, new surface Ag's (fetal or viral)
  - changes in membrane transport and components
  - lower cAMP levels

DNA Tumor Viruses

- Papilloma viruses--
  - probably transforms all cells they infect: some result in benign tumors and others malignant.
  - DNA either integrated or as episome in 5-200 copies per cell.
  - Only 10% of DNA is transcribed in transformed cells.
  - 36 serotypes of human papilloma virus
  - many cause benign warts, some progress to malignant, (25%) especially in areas high in sunlight (anogenital warts [*Condylomata acuminata*])
  - 100% of cervical biopsies that contain precancerous changes contain HPV antigens

Polyoma Viruses

- When injected into newborn mice it causes tumors, rarely in adults. First isolated from apparently normal cultures of monkey kidney cells. The host in which it causes tumors is the baby hamster.
- 2 human polyoma viruses: BK & JC (patients, 1971). Most adults have antibodies to these. Neither virus causes tumors in humans, but urine extracts cause tumors in other animals (nerve tissues & brain)

Transformation by Polyoma Viruses

- Cells transformed if non-permissive of if they are infected with defective particles (if virus can multiply it will not transform).
- Transformation requires high infectivity (10^6-10^7 particles per cell). # of integrated genomes 1-50.
- Viral DNA does not replicate but does express itself:
  - not entire genome transcribed
  - early genes expressed-- Large T and small T Ag's

Large and Small T Ag's

- Large T--
  - stimulates host cell DNA synthesis. Must do this to transform cell
  - responsible for growth in soft agar
  - responsible for low serum requirements
  - small portion of it is exposed on the transformed cell membrane as a "*transplantation antigen*", and Ab's synthesized against it and may be protective against subsequent polyoma viral infection.
  - Lg T Ag associated with cellular phosphoprotein, especially of p53 protein, which is stimulated 25-50 fold (involved in normal cell proliferation)

Large and Small T Ag's

- Small T antigen:
  - necessary for loss of contact inhibition
  - causes degradation of intracellular actin network (characteristic of transformed cells)
  - increases tyrosine phosphorylation (important in cancer process???)

Adenovirus Transformation

- Human Serotypes 12, 18, and 31 highly oncogenic when injected into newborn hamsters.
- Other serotypes weakly oncogenic or not at all.
- Cells transformed with adenovirus type 12 express less class I MHC on surface (allow for escape from immune killing).
- Adenovirus has a portion of genome integrated, but not entire genome

Herpes Viral- Cell Transformation

- In humans 3 herpes type viruses involved in cell transformation: Epstein-Barr (EBV), Herpes Simplex type 2 (HSV 2), and human cytomegalovirus (HCMV)
- EBV- Burkitt's lymphoma- common in East Africa and New Guinea (malerial contribution) but rare in other parts of the world. Primarily in children 5-12. No infectious virus in cells.
  - 80% of adults have Ab's to EBV
  - Infects B lymphocytes
  - 2-200 episomal closed circles in infected cell nucleus, and only a few integrated

EBV

- Causes Burkitt's lymphoma-
  - All BL tumor cell lines contain a reciprocal exchange between the terminal portions of chromosomes 8 & 14 (sometimes between 8 and 2 or 22). [14=Ig H, 2=Ig kappa, 22=Ig lambda]
  - The portion of #8 that is exchanged contains the *c-myc* gene (a proto-oncogene). This is normally silent, but when exchanged it comes under control of active promoters (Ig genes on chromosomes 14 and 22)
- Thus, EBV initiates translocation and replication of B cells and malaria suppresses immune function and malignant transformation occurs.

Herpes Simplex Virus

- Association between HSV 2 and cervical cancer
- two sets of gene sequences that are able to transform cells that are separated by 20,000 BP in non-transformed cells, but are contiguous in transformed cells.

- Hepatitis B Virus-
  - only known host is human
  - 200 million currently infected with HBV
  - causes hepatic cancer (hepatoma cells contain integrated viral genome)

Proto-oncogenes & Oncogenes

- Proto-oncogenes- cellular genes/normal function, as long as they are controlled. They are primarily growth control genes (DNA replication control genes, growth factor genes, phosphorylation genes, etc….)

- Oncogenes- 40 cancer causing genes known. Each has close homology with proto-oncogenes (maybe one BP change?). Under control of different regulatory mechanisms than normal.

Proto-oncogenes & Oncogenes

- How proto-oncogenes incorporated into viral genome unknown.
- 6 Groups of oncogenes-
  - Peripheral Membrane Proteins with Tyrosine-specific protein kinase activity
  - Transmembrane receptors with tyrosine-specific protein kinase activity
  - Cytoplasmic serine/threonine-specific protein kinases
  - Plasma membrane bound GTP-binding proteins
  - Growth Factors
  - Nuclear DNA binding proteins

TABLE 8–4. ONCOGENES AND PROTO-ONCOGENES

Oncogene	Virus	Protein Encoded by Oncogene	Protein Encoded by Proto-oncogene
Peripheral Membrane Proteins with Tyrosine-specific Protein Kinase Activity			
v-src	Rous sarcoma virus, B77 ASV	$pp60^{v\text{-}src}$	$pp60^{c\text{-}src}$
v-abl	Abelson murine leukemia virus	$pp160^{v\text{-}gag\text{-}abl}$	$pp145^{c\text{-}abl}$
v-fes/fps	Snyder-Theilen FeSV; class II ASVs including Fujinami sarcoma virus	$pp85^{v\text{-}gag\text{-}fes}$ $pp130^{v\text{-}gag\text{-}fps}$	$pp92^{c\text{-}fes/fps}$
v-yes/fgr	Y73 Esh sarcoma virus Gardner-Rasheed FeSV	$pp90^{v\text{-}gag\text{-}yes}$ $pp70^{v\text{-}gag\text{-}fgr}$	$p55^{c\text{-}yes/fgr}$
v-kit	Hardy-Zuckerman FeSV	$pp80^{v\text{-}gag\text{-}kit}$	?
Transmembrane Receptors with Tyrosine-specific Protein Kinase Activity			
v-erbB	Avian erythroblastosis virus (AEV)	$P45^{v\text{-}erbB}$	EGF receptor: 150K
v-fms	McDonough FeSV	$P140^{v\text{-}gag\text{-}fms}$	Macrophase colony stimulating factor (CSF1) receptor: 170K
v-ros	UR2 ASV	$P68^{v\text{-}gag\text{-}ros}$	$P66^{c\text{-}ros}$
v-erbA	AEV	$P75^{v\text{-}gag\text{-}erbA}$	Thyroid hormone receptor: 70K
Cytoplasmic Serine/Threonine-specific Protein Kinases			
v-mos	Moloney murine sarcoma virus (Mo-MSV)	$P37^{v\text{-}mos}$	$P41^{c\text{-}mos}$
v-mil-raf	MH2 ASV; Moloney murine sarcoma virus strain	$P100^{v\text{-}gag\text{-}mil}$ $P75^{v\text{-}gag\text{-}raf}$ and $P90^{v\text{-}gag\text{-}raf}$	$P72^{c\text{-}mil/raf}$
Plasma Membrane-associated GTP-Binding Proteins with GTPase Activity			
v-ras	Harvey and Kirsten murine sarcoma virus (Ha- and Ki-MSV)	$P21^{v\text{-}ras}$	$P21^{c\text{-}ras}$
Growth Factor			
v-sis	Simian sarcoma virus (SiSV)	$P28^{v\text{-}sis}$	$P32^{c\text{-}sis}$-PDGF
Nuclear DNA-binding Proteins			
v-myc	Avian myelocytomatosis virus (MC29I	$P110^{v\text{-}gag\text{-}myc}$	$P62^{c\text{-}myc}$
v-myb	Avian myeloblastosis virus (AMV)	$P45^{v\text{-}myb}$	$P75^{c\text{-}myb}$
v-fos	FBJ murine osteosarcoma virus	$P55^{v\text{-}fos}$	$P55^{c\text{-}fos}$
v-ets	E26 avian acute leukemia virus	$P135^{v\text{-}gag\text{-}myb\text{-}ets}$	$P54^{c\text{-}ets}$, $P75^{c\text{-}ets}$
v-ski	SKV770		

Proto-oncogenes & Oncogenes

- Proto-oncogenes- expressed normally in many cells at different stages of development.
  - *C-myc* and *c-myb* expressed in proliferating cells and *c-fos* in differentiating cells.
  - *C-src* expressed abundantly in brain, *v-src* has increased kinase activity
  - several proto-oncogenes expressed in increased amounts in cancer cells
  - proto-oncogene may be under control of its normal promotor, or may have been translocated to be under control of a different promoter.

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TABLE 8-6. PROTO-ONCOGENES EXPRESSED IN NORMAL CELLS

Proto-oncogene	Normal Cells
c-src	Neural tissue (neurones, astrocytes) Blood platelets
c-fms/fps	Myeloid cells, macrophages, and granulocytes
c-fms	Bone marrow and blood cells, macrophages, spleen Differentiating monocytes and other cells
c-mos	Mouse embryo, testes, ovaries
c-Ha-ras	Normal bladder epithelium
c-sis	Activated monocytes
c-myc	Most dividing cells; expression changes during cell cycle Wounded cell monolayers Quiescent fibroblasts stimulated with epidermal growth factor Differentiating erythroleukemia cells Late embryonic cerebellum
c-fos	Differentiating cells including bone marrow cells, macrophages, monocytes, myelocytes Cells stimulated with growth factors Late gestational extraembryonic tissue
N-myc	Mouse embryos, adult brain
c-myb	Early embryos, hematopoietic cells

TABLE 8-7. PROTO-ONCOGENES EXPRESSED IN INCREASED AMOUNTS IN HUMAN TUMORS

Proto-oncogene	Tumor
c-abl	Chronic and acute myelogenous leukemia (CML and AML)
c-fms/fps	AML
c-yes/fgr	Lymphomas, including Burkitt's lymphoma
c-erb/erf	Glioblastoma
c-Ki-ras	Giant lung cell carcinoma, primary hepatocarcinoma, pancreatic cancer T cell ALL
c-Ha-ras	Bladder cancer Primary hepatocarcinoma
c-N-ras	AML and others
c-sis	Neuroblastomas
c-myc	Colon adenocarcinoma Small lung cell carcinoma Giant lung cell carcinoma Primary hepatocarcinoma Acute leukemias
c-N-myc	Neuroblastomas, teratocarcinoma stem cells Small lung cell carcinoma
c-L-myc	Small lung cell carcinoma
c-myb	Acute leukemias
c-fos	Myelomonocytes and monocytic leukemias

Proto-oncogenes & Oncogenes

- Mechanisms of action of proto-oncogenes:
  - increase in the concentration of the gene product (viral oncogenes become activated when inserted into active genes)
  - expression of gene in an inappropriate cell type (ectopic expression). Results from mutation in regulatory region of the gene or infection with oncogenic viruses
  - unscheduled expression of a proto-oncogene that normally expresses gene in a temporally controlled way
  - alterations in the proto-oncogene product might confer oncogenicity (1 amino acid change)

AIDS

- AIDS (Acquired Immune Deficiency Syndrome) is a syndrome, not a single disease
- 1st cases of AIDS-related Pneumocystis carnii pneumonia reported by CDC in June 1981
- Luc Montagnier discovered the AIDS virus in 1983
- AIDS patients may have many diseases, and all of these are referred to as a syndrome. The severity of the expression and the different diseases reflects the status of the persons immune system.
- AIDS IS NOT TRANSMITTED, THE VIRUS IS!

AIDS

- There were several different names given to the virus (HTLV III, etc…) but to limit confusion it was given name "human immunodeficiency virus (HIV)
- In 1985 a 2nd type of HIV discovered in West African protitutes (HIV-2) and 1st confirmed case of HIV-2 in US was in 1987 (beginning of 1998 there were 72 cases of HIV-2 in US). Not as severe disease as HIV-1, and may give resistance to HIV-1 infection.
- HIV-2 more closely related to SIV

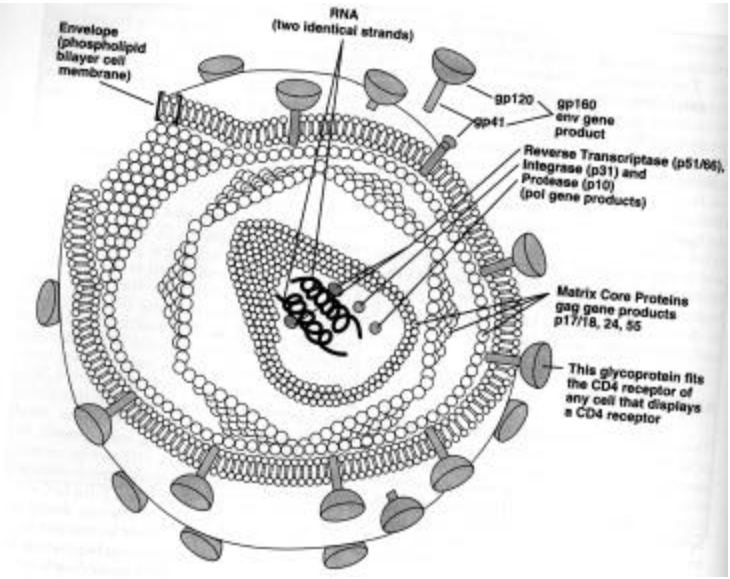


FIGURE 3-1 Human Immunodeficiency Virus. It infect cells by a process of membrane fusion that is mediated by its envelope glycoproteins (gp120-gp41, or Env) and is generally triggered by the interaction of gp120 with 2 cellular components: CD4 and a coreceptor belonging to the chemokine receptor family. The virus is a sphere measuring 1,000 A or 1/10,000 mm in diameter. The truncated cone-shaped core in a spherical envelope is the dominant feature. In this diagram the virus has been sectioned to better visualize its internal structure. The membrane of HIV is derived from the host cell. HIV gains the membrane while "budding" out or exiting the cell. Each free HIV leaves a hole in the cell membrane. The membrane, acquired from its host cell, consists of two lipid (fat) layers impregnated with some human proteins, for example Class I and Class II human lymphocyte antigen complexes important for controlling the immune response. The external viral membrane also contains molecules of viral glycoproteins **(gp)**— a sugar chain attached to protein. Each glycoprotein appears as a spike in the membrane. Each spike consists of two parts: **gp41** which extends through the membrane; and **gp120** which extends from the end of gp41 to the outside and beyond the membrane (the numbers 41 and 120 represent the mass of the individual gps in thousands of daltons). As a complete unit, gp41 plus gp120 is called gp160. These two membrane or **envelope** proteins play a crucial role in binding HIV to CD4 protein molecules found in the membranes of several types of immune system cells. The gp160 precursor is cleaved into envelope (gp120) and transmembrane (gp41) proteins in the cell's Golgi compartment. The HIV envelope complex is transported via vesicles to patches in the outer cell membrane. Full-length HIV RNA is complexed with capsid proteins and the nucleocapsid is transferred to the cell surface membrane at envelope-containing sites. The binding of gp to CD4 receptors makes such immune system cells vulnerable to infection. Other HIV proteins are located and described in this figure.

Within the cone-shaped core there are two identical strands of viral genomic RNA, each coupled to a molecule of transfer RNA (tRNA) that serves as a primer for reverse transcription of viral RNA into viral DNA. HIV RNA is 9,749 nucleotide bases long. Also present with the RNA are an **integrase**, a **protease**, and a **ribonuclease enzyme**. The released virus is processed internally by HIV protease to form the characteristic dense lentivirus core. Most HIV appear to have initiated DNA synthesis prior to completion of budding and maturation. Actual maturation of HIV takes place after it buds out of the cell (see Figure 4-11).

Name(s)	Molecular Mass (kDa)	Function
GAG (group antigen)	p17	Matrix (protection)
	p24	Capsid (protection)
	p7	Nucleocapsid (protection)
POL (polymerase)	p10	Protease (enzyme)
	p32	Endonuclease (enzyme)
	p66, p51	Reverse transcriptase (enzyme)
ENV (envelope)	gp120	Surface envelope
	gp41	Transmembrane envelope
tat (transactivator protein)[a]	p14	Transactivation of all HIV proteins
rev (differential regulator of expression of virus protein)	p19	Increase production of structural HIV Protein; Transport of spliced/unspliced RNA from nucleus to cytoplasm
vif (virus infectivity factor)	p23	Required for ineffectivity as cell-free virus
nef (negative regulator) factor)	p27	Retards HIV replication
vpr (virus protein R)	?	Triggers cells steroid production to produce HIV
vpu (virus protein U)	p16	Required for efficient viral replication and release-budding

AIDS

- Definitions:
- **in 1982 there was no single characteristic of AIDS that would allow for a useful definition for surveillance purposes. No immunological testing available**
  - The presence of a reliably diagnosed disease at least moderately predictive of cellular immune deficiency and
  - the absence of an underlying cause for the immune deficiency of for reduced resistance to the disease
- **1983 and 1985 definition altered to include new diseases found in AIDS patients**
- **AIDS refers to the onset of life threatening illnesses as a result of HIV disease that results from an HIV infection. AIDS is the end state of a disease process which may have been developing for 5-15 years**

AIDS

- In 1993 the definition of AIDS was revised again to include women and to include all persons with a T4 cell count of less than 200 cells/ul of blood, i.e., a T4 lymphocyte percent less than 14% of total lymphocytes. (See table next slide)
- During 1993, 990 children (<13 years old) were reported with AIDS, a 21% increase from 1992.
- Some people do not believe that AIDS is caused by a virus (Willner- stabbed himself with infected needle, Duesberg- virologist)

AIDS

- AIDS has satisfied all of Koch's Postulates:
  - 1. Causative agent must be found in all cases of the disease (It is.).
  - 2. It must be isolated form the host and grown in pure culture (It has).
  - 3. It must produce the original disease when introduced into a susceptible host (It does).
  - 4. It must be found in the experimental host so infected (It is.).

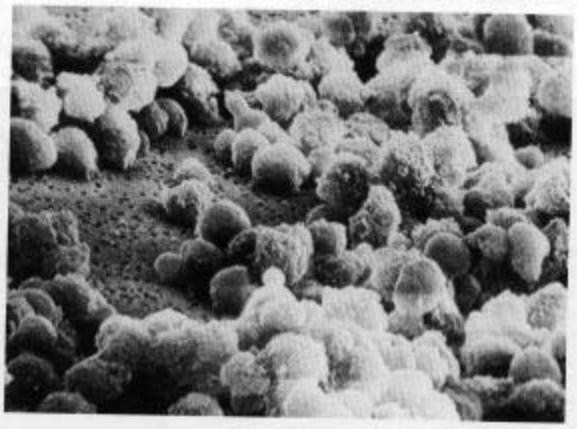


FIGURE 4-7 Production of HIV. Each dark hole in the T4 cell membrane represents the emergence of one new HIV that came off the membrane and is loose in the body. Each T4 cell produces about 250 HIV before it becomes nonfunctional. Each HIV in this photograph is genetically different from any other HIV being released from this T4 cell and most likely genetically different from any other HIV in the body. (*Source: The CDC, Atlanta, GA*)

AIDS Attachment and entrance into cells

- Major envelope protein is gp 120
  - contains a constant region (constant from virus to virus) and a variable region
  - variable region made up of five domains, each containing a separate sequence of AA's. Has extensive number of AA substitutions.
  - Each variable region given name V1---V5
  - V3 most variable. Helps gp120 to lock onto CD4 (Ab to V3??)
  - HIV circulates in blood until it bumps into a T helper cell with CD4 antigen on membrane

AIDS Attachment and entrance into cells

- Other Receptors for entrance into cell:
  - CD4 alone is sufficient for binding, but not sufficient for fusion and penetration
  - May 1996-- Berger found cell receptor they called FUSIN
  - August 1996-- Bleul identified a chemokine, CXC stromal cell-derived factor-1 (SDF-1) that binds to the FUSIN receptor and blocks HIV entry= CXCKR-4 is the name of the chemokine (C stands for cysteine and X is any other AA)

AIDS Attachment and entrance into cells

- There are 7 known β-chemokine receptors: Their function is to attract macrophages and other immune cells to the site of inflammation
  - CKR-5 is a receptor for 3 β-chemokines called RANTES, MIP-1α , and MIP-1β (macrophage inflammatory protein).
  - RANTES (Regulated-upon-Activation Normal T Expressed and Secreted)
  - All of these are produced by $CD8^+$ cells and inhibit HIV replication

AIDS Attachment and entrance into cells

- T-tropic HIV use FUSIN receptor to enter T cells
- M-tropic HIV use CKR-5 receptor to enter macrophages
- M-tropic HIV strains occur in greatest number early on after HIV infection, and then later during disease the predominant HIV strain shifts to T-tropic viruses
- Other chemokine receptors also found (CKR-2 and CKR-3) that allow entry into non-CD4 cells (brain and microglial)

AIDS Attachment and entrance into cells

- 95% of HIV exposed people are susceptible to HIV infection and HIV disease progression.
  - Some people have been found to have a deletion of 32 nucleotides in the gene that produces the CKR-5 receptors on macrophages, and this mutation prevents infection only with the strain of HIV that is transmitted sexually and is prevalent in the US and Europe
  - This mutated gene is homozygous and present in 1% of whites of European descent, but is absent in people from Japan and Central Africa. 20% of whites are heterozygous. (may have blocked plague bacteria)

AIDS: Means by which T cells are lost

- 1. Not yet fully understood
  - PCR has shown that one in every 10-100 T4 cells is HIV infected in an AIDS patient, but how the cells are killed and depleted is a mystery since viral replication cannot account for the massive killing
  - T cells and macrophages act as reservoirs for the virus

AIDS: Means by which T cells are lost

- Filling CD4 receptor sites-- gp120 and gp160 may become free of envelope and bind to CD4 thereby preventing T cell function. These cells do not have to be infected with virus and antigen binding may stimulate cytotoxicity
- Syncytia formation-- fusion of infected cells with non-infected cells to form giant multinucleated cells. Increases cytopathic effects.
- Superantigens-- bacterial or viral Ag's that are able to bind to a large number of T4 cells rather than one specific cell (response in 5%-30% of cells rather than 0.01%). Superantigen stimulation by HIV lead to anergy or deletion of a substantial % of T4 cells

AIDS: Means by which T cells are lost

- Apoptosis-- programmed cell death: HIV may induce by cross-linking CD4 Ag's that trigger activation of TCR and apoptosis.
- Cellular transfer of HIV-- If virus replicates it will kill T4 cell.
- Autoimmune mechanisms-- HIV tricks immune system into killing itself by producing cross-reacting antibodies
- Co-factors may help deplete T4 cells-- nutrition, stress and infectious organisms (mycoplasma, drugs, excessive exercise, herpes??) might accelerate HIV expression after infection.

AIDS: Means by which T cells are lost

- Role of macrophages:
  - important in spreading virus to target cells.
  - Probably infect macrophage first
  - macrophages probably transport virus to brain

- Prior to progressive decline in T cells and the development of AIDS, there is a symptom free period which may last for >10 years, and scientists thought that this represented a latent period w/o replication. There is no latent period, but a continuous struggle between virus and immune system in which balance slowly shifts in favor of virus. Immune system is functioning!

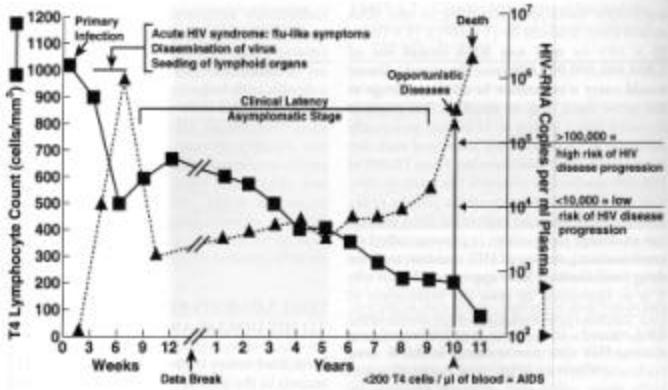


FIGURE 4-8 Clinical Course of HIV Disease as Related to T4 Cell Count and Level (number) of HIV-RNA Copies (viral load) in Plasma. During primary infection (the time period between infection and development of HIV antibody), HIV RNA levels spike (*triangles*) and HIV disseminates (*spreads*) throughout the body. This is followed by an abrupt drop in measurable HIV RNA in the blood (probably due to the production of HIV antibody), followed by a steady rise in HIV RNA until death. Over these same time periods, there is a continuous loss of T4 cells (*squares*). Note that the asymptomatic stage can be quite long—on average about 10 to 12 years, at which time T4 cell counts drop and HIV RNA copies increase to levels where there is high risk of opportunistic infections.

AIDS: Means by which T cells are lost

- In healthy individual 5% of T4 cells regenerated per day to replace lost cells (about 1.8 billion/day)
- AIDS patients with the lowest T cell counts had the highest rate of production (therefore, patient is trying to compensate, even at the end).
- Ho suggested that it may be better to plug the loss of cells rather than trying to increase replacement since replacement rates may be at maximum already
- HIV undergoes 3000-5000 replication cycles in 10 years producing a minimum of 10^{12} HIV. Lots of opportunity for mutation (1/round of replication).

Biological Markers Related to Progression of AIDS

- p24 antigen: specific protein located in the core (inner layer) of HIV. A positive test for p24 means that the antigen production (viral replication) is exceeding the amount of antibody in blood (not good!). Occurs at beginning of infection and at end when immune decline is occurring.
- Beta-2 microglobulin- present on surface of all nucleated cells, and high amounts in blood means chronic illness with increased cell death

Biological Markers Related to Progression of AIDS

- T 4 and T 8 levels-- $T_{helper}/T_{cytotoxic}$ ratio
- Levels of HIV RNA in Blood (Viral Load)-- more RNA means more virus.
- Viral load measurements indicate the amount of current HIV activity. T4 cell counts indicate the degree of immunologic destruction.

Name	FDA Approved	Usual Daily Dosage	Cost/Day[a]
Nucleoside analogs (reverse transcriptase inhibitors)			
Zidovudine (AZT, ZDV; Retrovir)	March 1987	600 mg	$14.00
Didanosine (ddI; Videx)	October 1991	200–400 mg	$3.34
Zalcitabine (ddC; Hivid)	June 1994	2.25 mg	$7.50
Stavudine (d4T; Zerit)	June 1994	60–80 mg	$8.80
Lamivudine (3TC; Epivir)	November 1995	300 mg	$6.22
Abacavir (1592)	? (Glaxo Wellcome/Vertex)	600 mg	Phase III
Non-nucleoside compounds (nonnucleoside reverse transcriptase inhibitors)			
Nevirapine (Viramune)	June 1996	400 mg	$6.88
Delavirdine (Rescriptor)	April 1997	1.2 g	$7.40
DMP 266 (Sustiva)	?(Merck)	—	—
Protease inhibitor drugs[b]			
Saquinavir (Invirase)	December 1995 (Hoffman-La Roche)	1.2 g	$21.60
Ritonavir (Norvir)	March 1996 (Abbott)	1.2 g	$16.15
Indinavir (Crixivan)	March 1996 (Merck)	1.6 g	$16.39
Nelfinavir (Viracept)	March 1997 (Agouron)	2.25 g	$18.58
ABT-378	Expected January 1998 (Abbott)	—	Phase III testing
VX-478	? (Glaxo Wellcome/Vertex)	—	Phase III testing

Anti-HIV Therapy

- Must remove provirus from infected cells or it could become activated at any time. Can this be done??
- Nucleoside analog reverse transcriptase inhibitors- **incorporated into strand of HIV DNA newly synthesized and stops further synthesis**
- Non-nucleoside analog reverse transcriptase inhibitors (NNRTIs)-- **not incorporated into DNA but inhibit by binding non-competitively to RT**
- Protease Inhibitors-- **function early in HIV life cycle. Prevent cleavage of long polypeptide into functional molecules.**

Anti-HIV Therapy

- Resistance mutants found in people who have never been treated with antiviral drugs
- Combination therapy best!
  - Only one strand out of one thousand trillion (10^{15}) will carry resistance to all three drugs at the same time.
  - While this chance is small, remember that 1-10 billion genetically different RNA strands are produced each day in one individual, and there are from 10,000 to 100,000 nucleotide changes for each nucleotide of HIV's 9,749 nucleotides!! It is possible to get resistance to all 3 drugs at the same time

Anti-HIV Therapy

- Must start therapy early before replication is too fast.
- The cumulative viral load over time is a function of both the initial viral load and the rate of change of viral load (how fast are new HIV being made).
- Must keep initial infectious load **LOW**

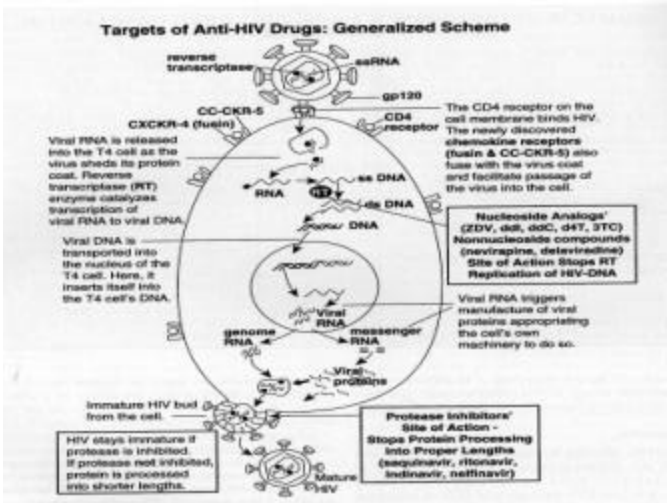


FIGURE 4-13 Diagram of Anti-HIV Therapy. The five FDA-approved nucleoside analogs, two nucleoside compounds, and four protease inhibitors are represented with respect to their anti-HIV activity. The nucleoside analogs act early after infection, while the protease inhibitors act later in the HIV life cycle, after viral proteins have been synthesized into long strands. Those strands of amino acids contain the individual HIV proteins that become functional after they are cut into their appropriate amino acid sequence lengths. **NOTE: The enzyme integrase is required for HIV DNA to enter human DNA. Drugs called INTEGRASE INHIBITORS are in development.**

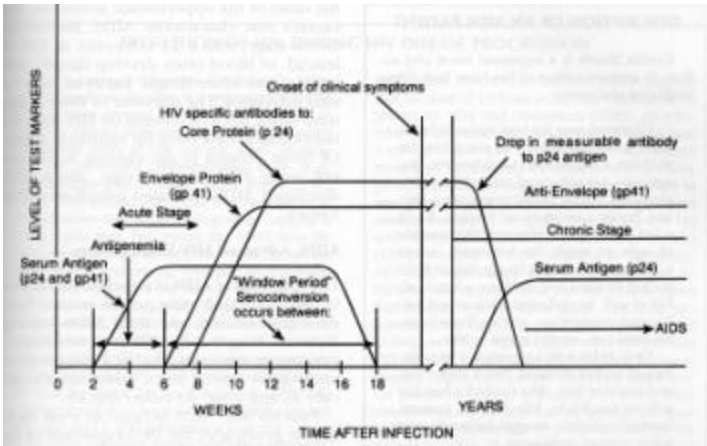


FIGURE 7-4 Profile of Serological Changes after HIV Infection. The dynamics of antibody response to HIV infection was determined by enzyme immunoassays (EIA). Note that during antigenemia, specific HIV proteins (antigens) can be detected before seroconversion occurs. Perhaps other HIV proteins will allow even earlier detection of HIV infection. Once antibodies appear, some antigens like p24 and gp41 disappear only to show up again later on. Note also that although antibody production is a sign that the immune system is working, in HIV-infected people, it is not working well enough. Although envelope and core protein antibodies are being produced as clinical illness begins, as the p24 antibody drops, the illness becomes more serious. *(Adapted from Coulis et al., 1987)*

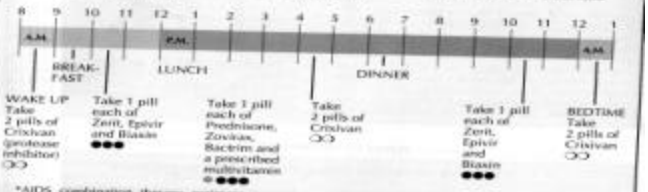
POINT OF INFORMATION 4.3 (continued)

BOX 4.5

THE NEW AIDS DRUGS: TIMING* IS CRUCIAL

Doctors are deciding that some people should not have access to new anti-HIV drugs because of the complex drug regimen to which HIV and AIDS patients must adhere. Failure to stick to the strict schedule could make the virus resistant to treatment and cause a potential health risk by creating drug-resistant strains of the disease. Here is how one patient described his daily medication schedule:

❍ Take without food ✱ Take with food ● Take with or without food



*AIDS combination therapy regimens are arguably the most demanding of any in medicine. Every day must be choreographed to coordinate such basic activities as working, eating, and sleeping with the consumption of a score of pills and liters of water. **One patient takes 72 pills daily.**

Some drugs require fasting; others must be taken with high-fat meals. Some are taken once a day, some twice, and some three times.

It is a rigorous treatment routine that wreaks havoc on already difficult lives.

One patient, who swallows 16 pills a day, said it took him months to devise a schedule that did not leave him hungry. Now he starts his day with a protease inhibitor, washed down with several glasses of water. Then he has to wait an hour for breakfast.

Between breakfast and lunch, he takes three more pills, and he must eat lunch by 2 P.M. so his stomach can be empty enough for his next protease inhibitor dose at 4:30 P.M. Then he must eat dinner by 6 P.M. to have a full stomach for the next round of drugs. His final challenge is to stay awake to take his last protease inhibitor at 12:30 A.M.

This patient said, "It's a good thing I can actually feel it prolonging my life, because the whole thing is so hard, especially with HIV, which makes you really forgetful."

For most patients, the drugs are also an unwelcome, unwelcoming reminder—with side effects like nausea, diarrhea, and kidney stones—that they have HIV (Source: *New York Times*, 3/2/97).

QUESTIONS: How can people high on drugs or those with AIDS dementia or those just too ill to function remember to take their drugs on time? In the proper sequence? or with the proper diet? How many have access to the proper diet, fat vs. non-fatty foods at given times?

STATEMENT: In some large cities like New York, some 75% of HIV/AIDS patients are on **protease inhibitors**. Not to be compliant (that is, taking the medication as prescribed, without failure) is to allow HIV to replicate and give rise to protease inhibitor mutants that may otherwise not occur! Such mutants will then be transmitted to other sex, many doctors say they have an ethical responsibility to withhold drug cocktails from some patients after judging them, not as people, but rather as compliant or noncompliant patients.

CLASS DISCUSSION: Is this a moral decision? Why? Why not?

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POINT OF INFORMATION 4.3 (continued)

Second, COMPLIANCE—The Achilles Heel of Anti-HIV Therapy: Skipping only a few pills can trigger the emergence of drug-resistant strains of HIV. Such a development could create a worse problem than the initial infection because the resistant virus could overwhelm the individual taking the drugs and anyone else to whom the individual transmitted the virus.

There is a present danger that the behavior of underdosing (not taking enough) or partial compliance (taking the protease inhibitor when they feel like it) or patients who modify their dosage regimens—**to extend their prescription**—may create HIV strains resistant to all currently available drugs. This would lead to an even more devastating AIDS pandemic! The major reason given by 202 HIV-positive people on combination anti-HIV therapy was, **I FORGOT!** (33%). Clearly, the degree of compliance to therapy affects treatment outcome. Andrea Barthwell (1997) reported that from 12 years of research, the clearest finding is that nonadherence is the norm in medical care.

Third, COSTS: AIDS is a disease of poverty. The majority of HIV-infected in the United States and worldwide will **never** get a first dose of a protease inhibitor or an AIDS cocktail. The cost of **just** protease inhibitors is between $12,000 and $20,000 per year! The cost to treat the 800,000 or so Americans would run about $6 billion a year!! Larry Kramer, a co-founder of Gay Men's Health Crises, states that the cost of his drugs to combat AIDS, **which do not include a protease**, amounts to about $19,000 a year; this does not include visits to his doctor or the batteries of blood tests that he routinely requires. And he is asymptomatic. A *New York Times* article in 1997 estimated that drugs for someone with symptomatic AIDS cost about $70,000 a year; in response, a New York University adjunct law professor and gay-rights advocate, wrote a letter to the editor saying that his drugs cost $84,000 a year using protease inhibitors; the annual drug cost can exceed $150,000. At these prices, how many of the nation's 650,000 to 900,000 HIV-infected will be able to afford proper HIV therapy? **AT THESE PRICES PEOPLE HAVE TO CHOOSE WHETHER TO PAY RENT, BUY FOOD, OR PAY FOR THEIR MEDICINE—SOME CHOICE!**

Fourth, SIDE EFFECTS: Because of the short time period from development to approval, many of the drug interaction studies with the protease inhibitors are unpublished or have yet to be performed; therefore, it is difficult to make rational decisions about how to use many of those drugs when using protease inhibitors. Since people with advanced HIV disease use perhaps as many as 15 drugs for the treatment of opportunistic infections and for the treatment of HIV itself and the psychiatric problems that arise with a chronic fatal disease, significant drug interactions resulting in toxic effects are unavoidable.

For practicing clinicians it is more important than ever that **before adding a protease inhibitor to the therapeutic regimen**, the prescriber reviews the drug list of the patient. It is crucial to understand how those drugs are eliminated (renal vs. hepatic, and, if hepatic, conjugation vs. oxidation), so as to make rational choices about those drugs by altering doses as necessary to reduce the likelihood of toxic effects or loss of efficacy. For example, by May of 1997, 83 cases of anti-HIV protease inhibitor therapy-induced **hyperglycemia** (increased blood sugar levels) and **diabetes** were reported to the CDC. All four FDA-approved PIs are involved. The average time of onset was 76 days after beginning treatment. At that time about 150,000 people were on protease inhibitors. Other metabolic problems recently encountered are high triglycerides and high cholesterol. There have been a number of anecdotal reports that people on **Crixivan** are gaining belly weight, or "**Crix belly**," up to 40 pounds or more rather quickly. There is, at the moment, a rather long list of drugs that should either **not be taken or taken in reduced amounts** when taking a protease inhibitor (Flexner, 1996; Gerber, 1996; Dubé et al., 1997).

Diseases

HIV Disease

Group 1: Acute infection—HIV antibodies absent; asymptomatic or if symptomatic mononucleosis-like symptoms which subside in most cases

Group 2: Asymptomatic infection—HIV antibodies present; eventually moves on to

Group 3: Persistent generalized lymphadenopathy; eventually moves on to group 4

AIDS

Group 4:

Subgroup A: Constitutional symptoms (previously called ARC, aids-related complex) and <200 T4 cells/μl of blood.

Subgroup B: Neurologic, symptoms (AIDS dementia complex, neuropathy)

Subgroup C

Category C-1: Secondary infections listed in the CDC surveillance definition for AIDS: *Pneumocystis carinii* pneumonia, recurrent pneumonia, chronic cryptosporidiosis, CNS toxoplasmosis, extraintestinal strongyloidosis, isosporiasis, esophageal candidiasis, cryptococcosis, histoplasmosis, *Mycobacterium avium intracellulare* or *Mycobacterium kansasii*, cytomegalovirus, chronic or disseminated herpes simplex, progressive multifocal leukoencephalopathy

Category C-2: Other specified secondary infections: oral hairy leukoplakia, multidermatomal herpes zoster, recurrent *Salmonella* bacterium, nocardiosis, pulmonary tuberculosis, oral candidiasis

Subgroup D: Secondary cancers: Kaposi's sarcoma, non-Hodgkin's lymphoma (small, noncleaved lymphoma; immunoblastic sarcoma), primary brain lymphoma

Subgroup E: Other conditions: lymphocytic interstitial pneumonitis, neoplasms, infections not previously listed