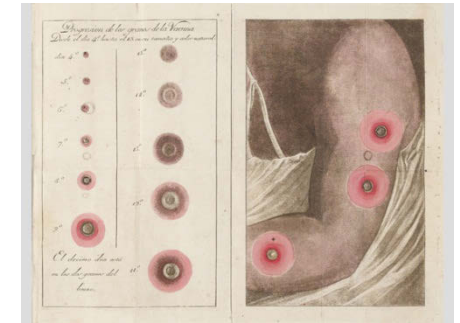
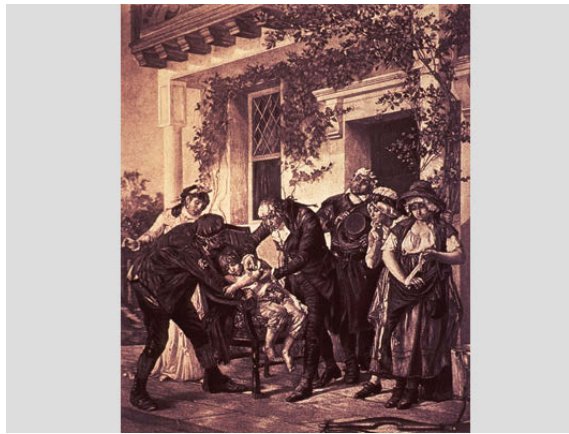




1157 BC



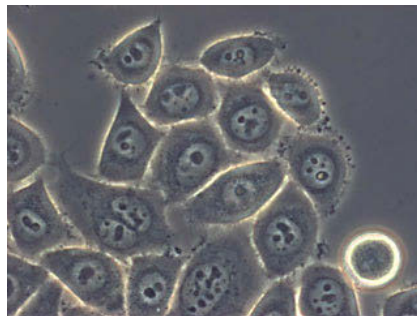
The marriage of Medicine and Cell Biology....

Ricardo Jose Gonzalez-Rothi, MD

Richard S. Nowakowski, PhD



1951



FLORIDA STATE UNIVERSITY
COLLEGE of MEDICINE

Upcoming “Marriage Series”

October 12: In Sickness and in Health: The Marriage of Cell Biology and Medicine

Richard S. Nowakowski, PhD, Chair Biomedical Sciences, and RJ Gonzalez-Rothi, MD Chair Clinical Sciences

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November 16: Is there a Pill I can take for this? Or more than you'll ever want to know about how drugs are developed.

Yanchang Wang, PhD Biomedical Sciences and John Blackmon, MD, Biomedical Sciences

What we wish to share with you today...

- Clinical presentation of two common individual and societally-devastating diseases
- How Physicians and Basic Scientists have collaborated purposely and/or serendipitously
- Vaccine development as the model

polios



myelos



itis

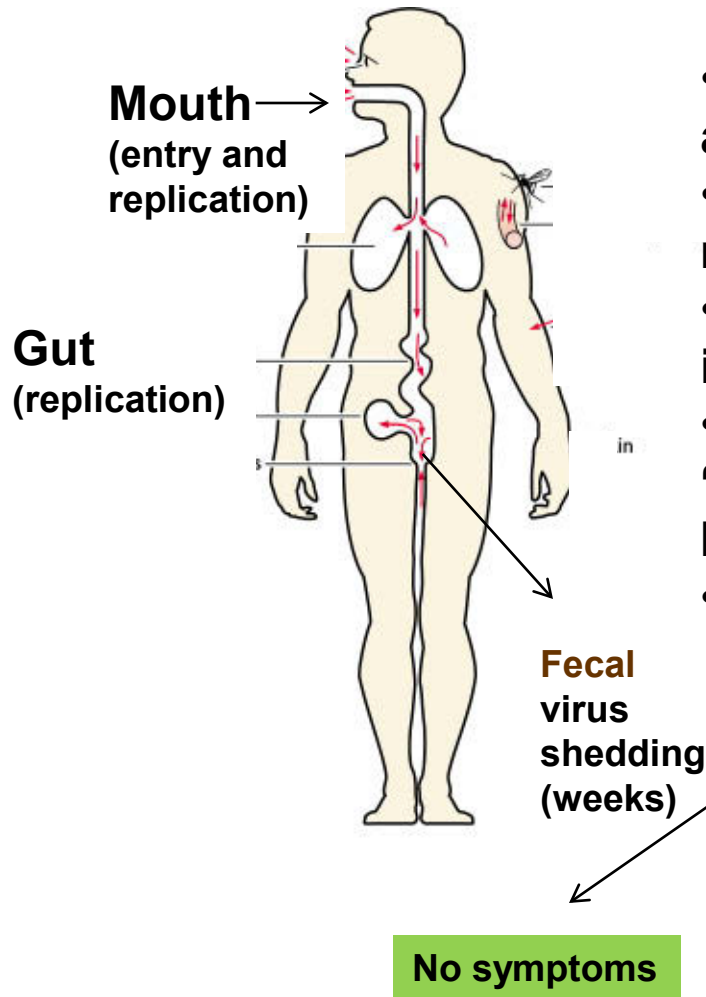


National Foundation for Infantile Paralysis



Poliomyelitis: the virus

“entero” (Gr. Intestine) virus (es)



- Virus enters mouth (orofecal spread), attaches to lining cells
- “kidnaps” lining cells’ “machinery”, replicates itself in mouth and gut
- Virus enters lymph nodes in throat, intestine wall and abdomen
- Sometimes virus spills into bloodstream “viremia”, on occasion enters nerves and brain/spinal cord lining (meninges)
- Incubation 6-20 days

No symptoms

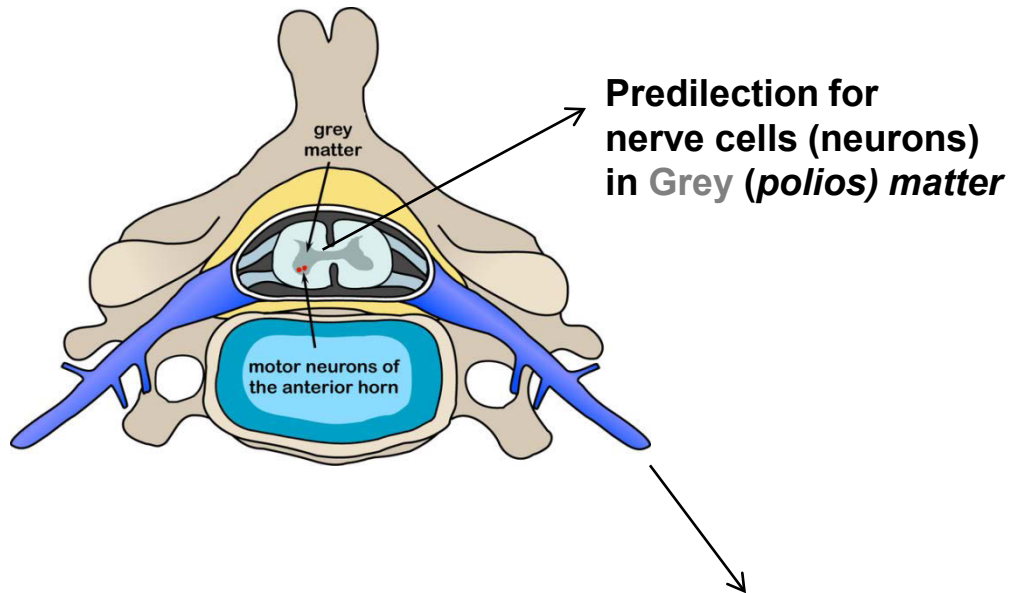
Mild illness

Major illness

Symptoms and signs

- Fatigue
- Fever
- Nausea, diarrhea,
- Headache
- Stiff neck
- Weakness in muscles, muscle pain, constipation, trouble urinating, breathing, difficulty swallowing, paralysis*
- Excruciating “hyper” sensation and pain

How it causes paralysis



→ Weakness, paralysis → contractures





The Birth of intensive care...



Last case of polio in the Americas

What is Cell Biology?

Cell biology is, for medicine, the most basic of the basic sciences.

Medical Treatments, Drugs, Devices, etc.

Preclinical Testing, Translational Medicine, etc.

Anatomy, Physiology, Immunology, etc.

Cell Biology, Molecular Biology, etc.

Physics, Chemistry, Math, etc.

The Cell is the Basic Building Block of Life

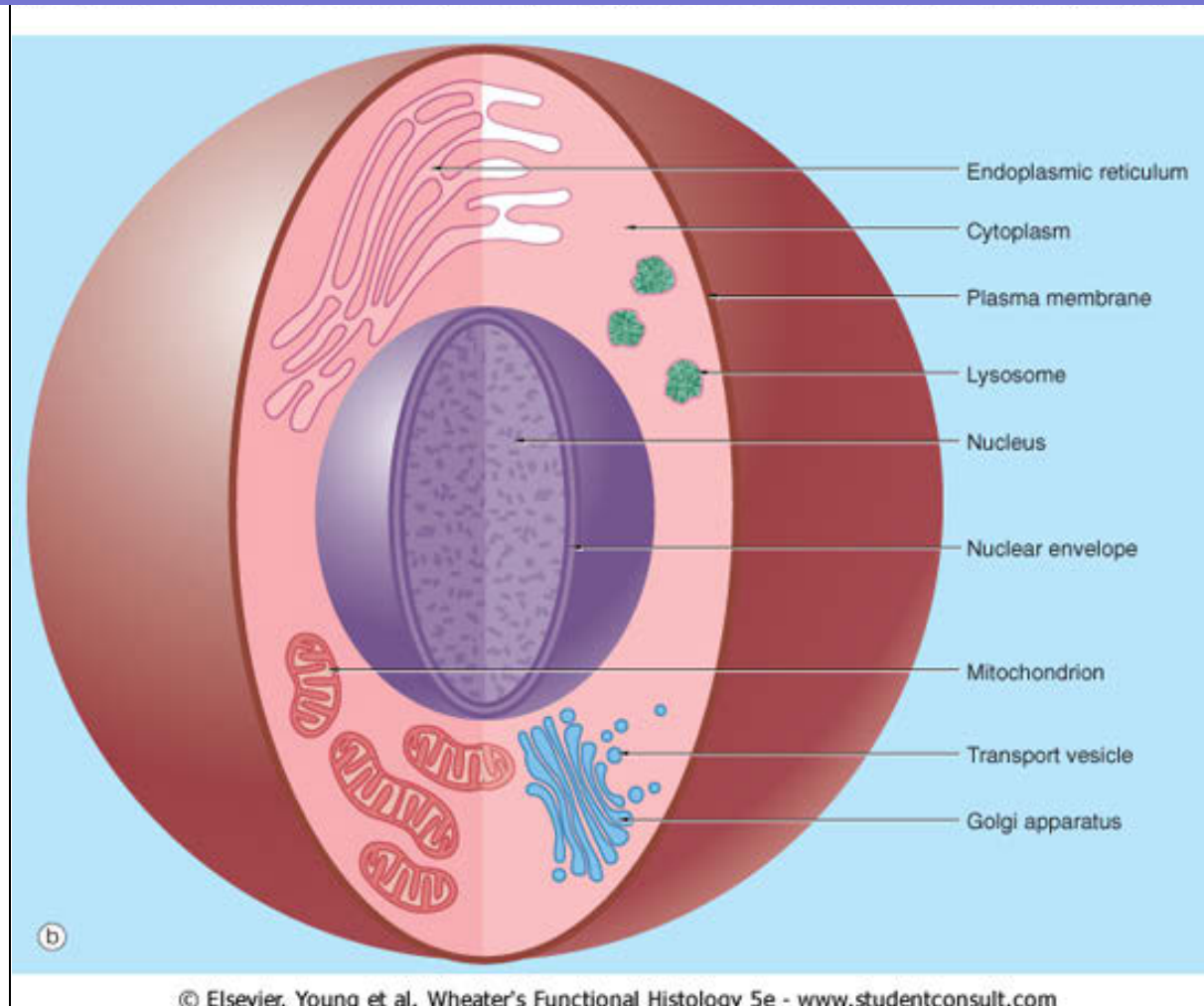


Figure 1.1 The cell (a) EM ×16 500 (b) Schematic diagram; C adjacent cells ER endoplasmic reticulum F collagen fibrils G Golgi apparatus IS intercellular space L lysosome M mitochondria N nucleus NE nuclear envelope PM plasma membrane V secretory vesicles

Real Cells are not Spheres!

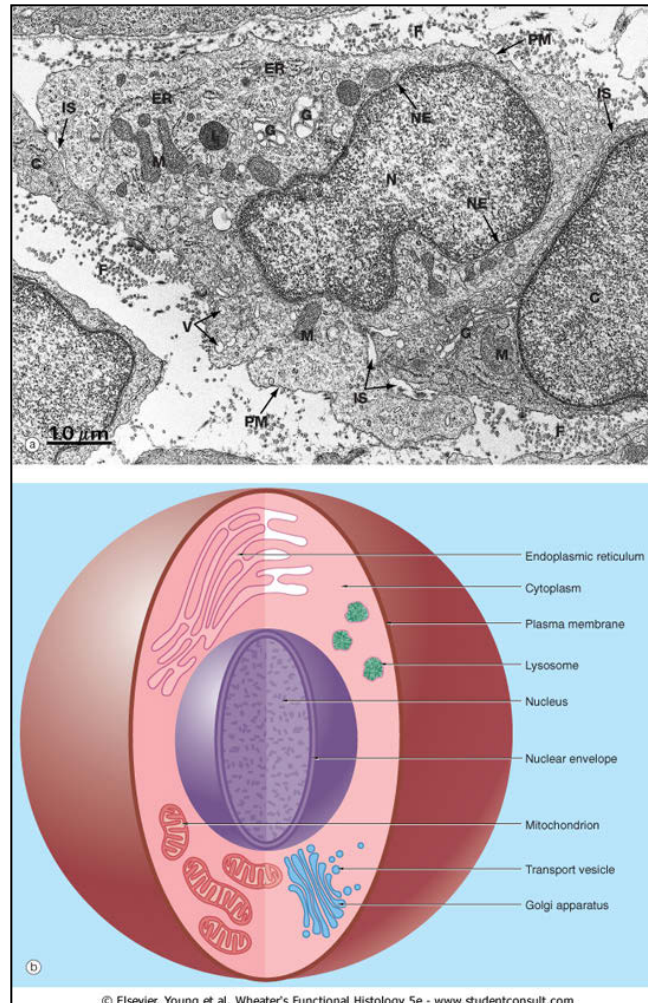


Figure 1.1 The cell (a) EM $\times 16,500$ (b) Schematic diagram; C adjacent cells ER endoplasmic reticulum F collagen fibrils G Golgi apparatus IS intercellular space L lysosome M mitochondria N nucleus NE nuclear envelope PM plasma membrane V secretory vesicles

Facts about Cells

- **10^{15} cells in a human (1,000,000,000,000,000) – more than 1,000 times the US National debt**
- **We all start as 1 cell**
- **Biggest human cell is the egg (ovum) which is ~100 μm in diameter (0.1 mm or about the same as the thickness of hair)**
- **Smallest cell is about 100 times smaller (1-2 μm in diameter)**
- **DNA has 4 billion bases**
- **All cells have the same DNA!**
- **Each cell contains 5-10,000 different proteins**

Cells Multiply by Dividing!

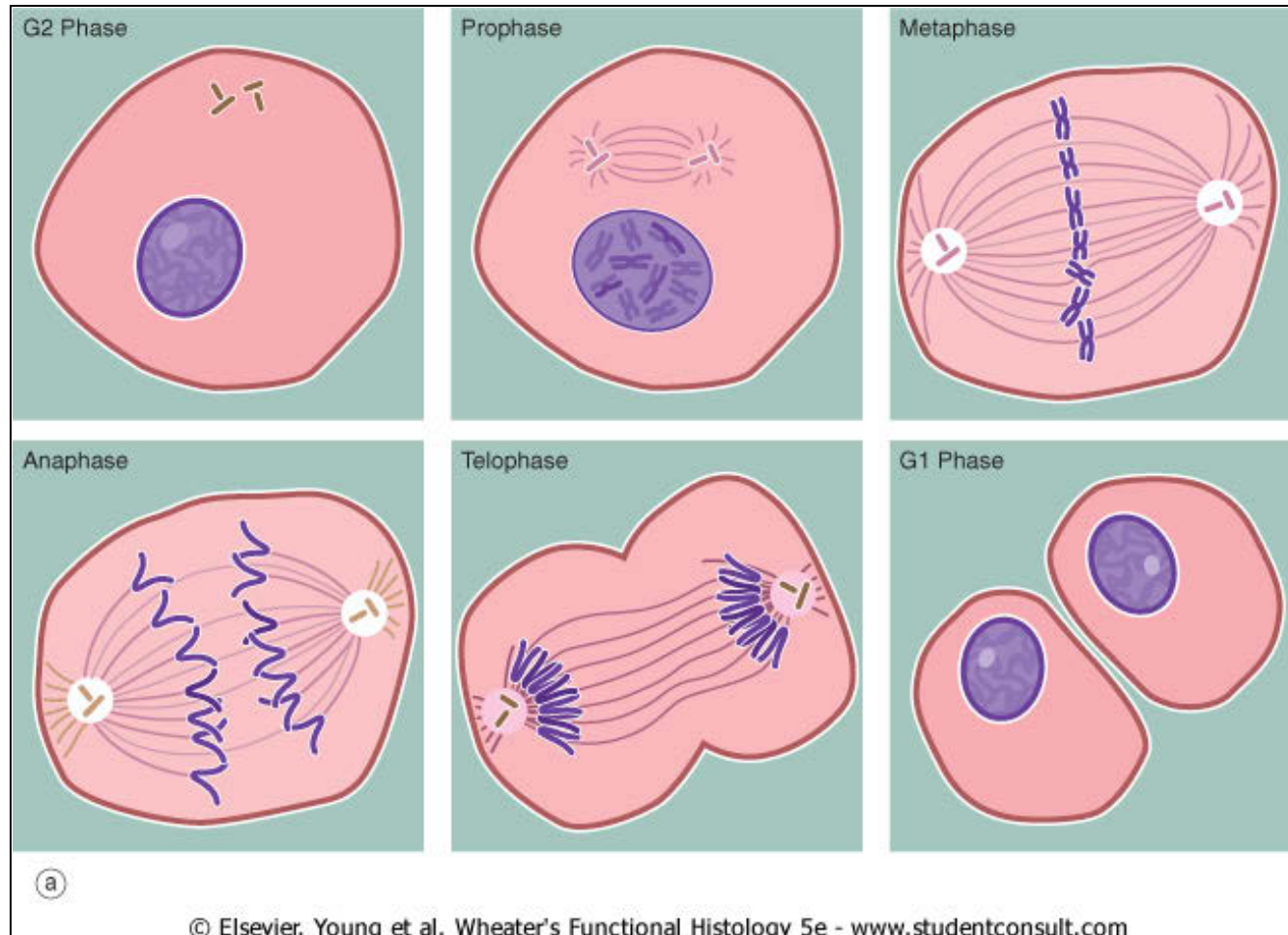
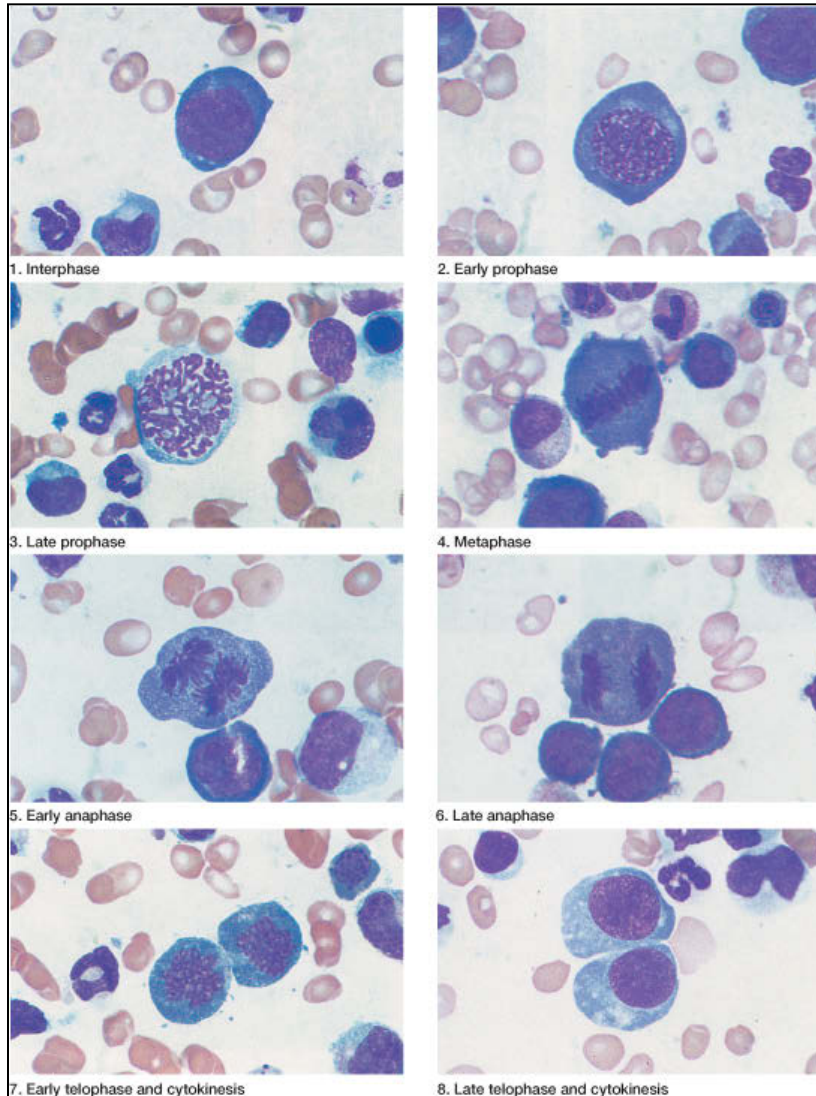


Figure 2.3 Mitosis (a) Schematic diagram (b) Mitotic series Giemsa ×800

Divisions Increases Cell Numbers FAST!

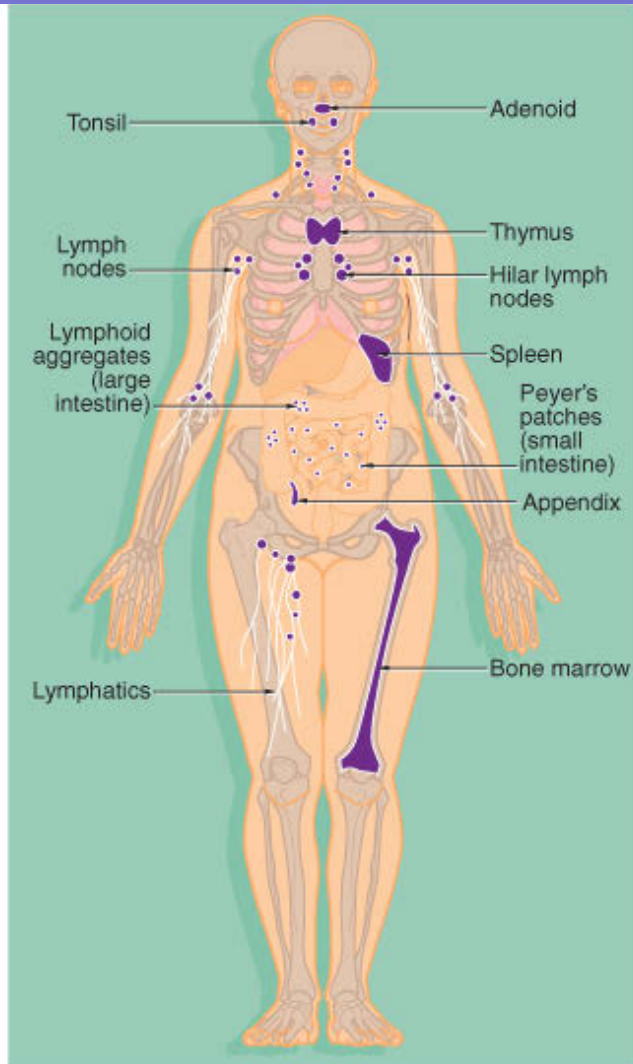


- 10 divisions → 1,000 cells (1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024)
- 20 divisions → 1,000,000 cells
- Each division in human is about 24-48 hours
 - Bacteria grow much faster
- Conclusion: to make 10^{15} cells is not hard – need about 50 divisions and 2 months

Brief History of Small Pox

- **1000 BC in India – vaccination with live small pox virus**
- **1790's Jenner uses cow pox – related virus – successfully**
 - **“Vaccine” is from “vacca” the latin word for “cow”!**
- **300 million deaths from small pox in 20th century alone → world-wide vaccination effort → 1979 small pox eradicated!**
 - **Stabilization of virus with peptone in 1940's enabled this (Collier, UK virologist)**

The Immune System



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Figure 11.1 The organs of the immune system; Ag antigen APC antigen presenting cell B B lymphocyte IL interleukin MAC membrane attack complex MHC major histocompatibility complex PA processed antigen slg surface immunoglobulin Tc cytotoxic T cell TCR T cell receptor Th T helper cell

Blood Cells and Immune Cells are Related

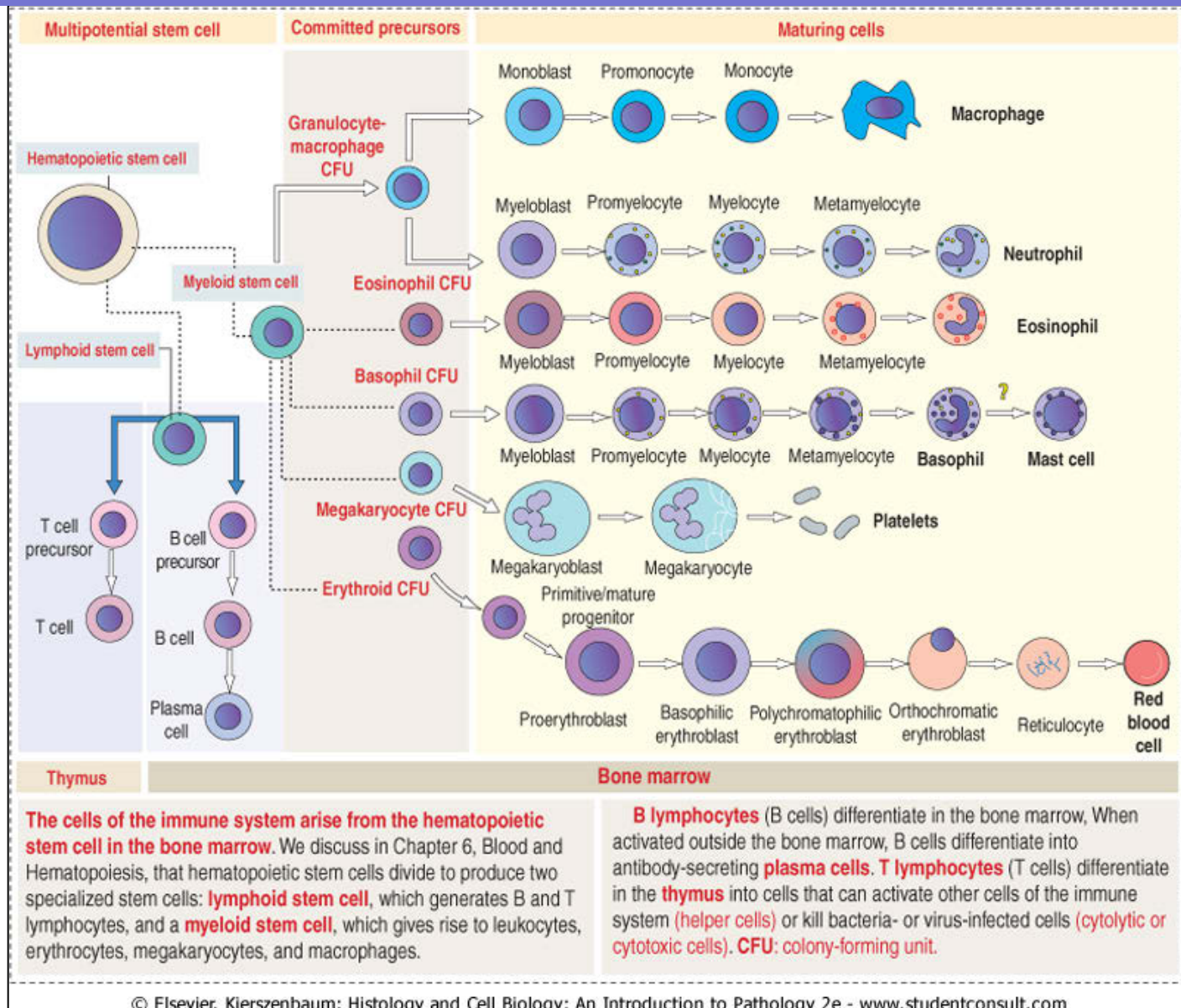
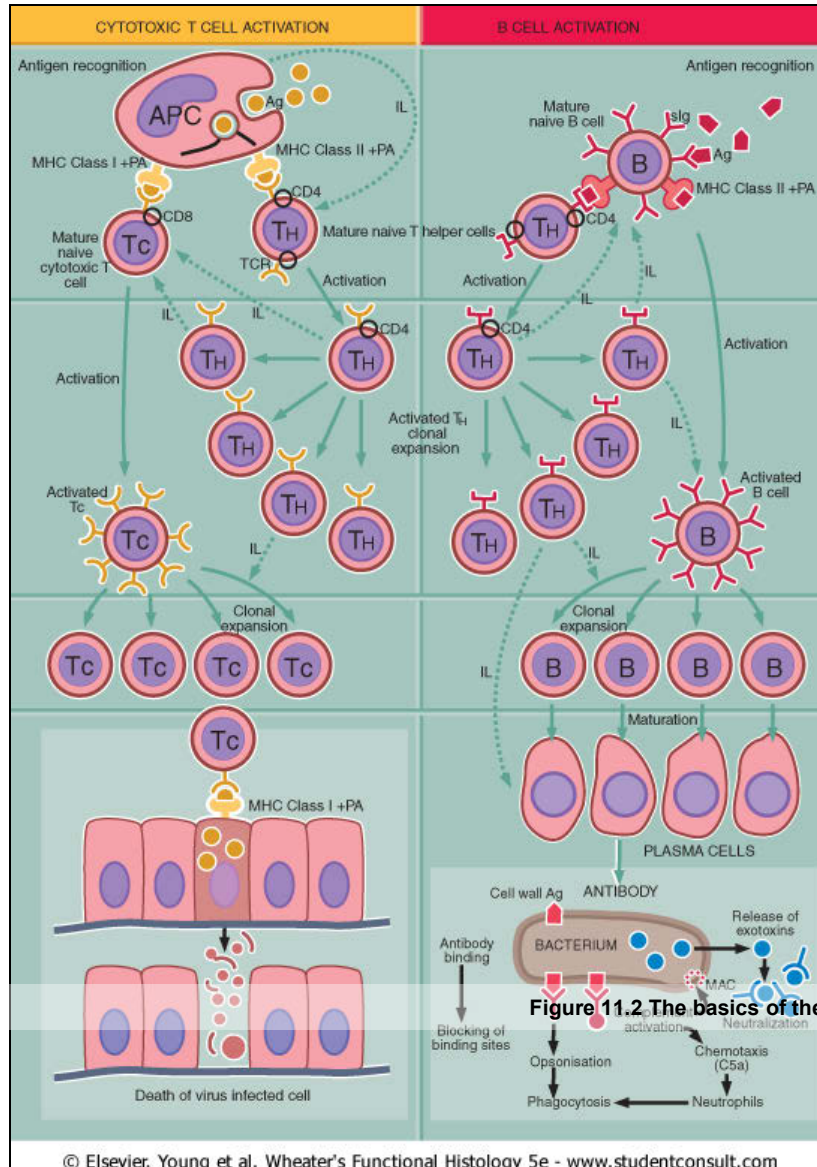
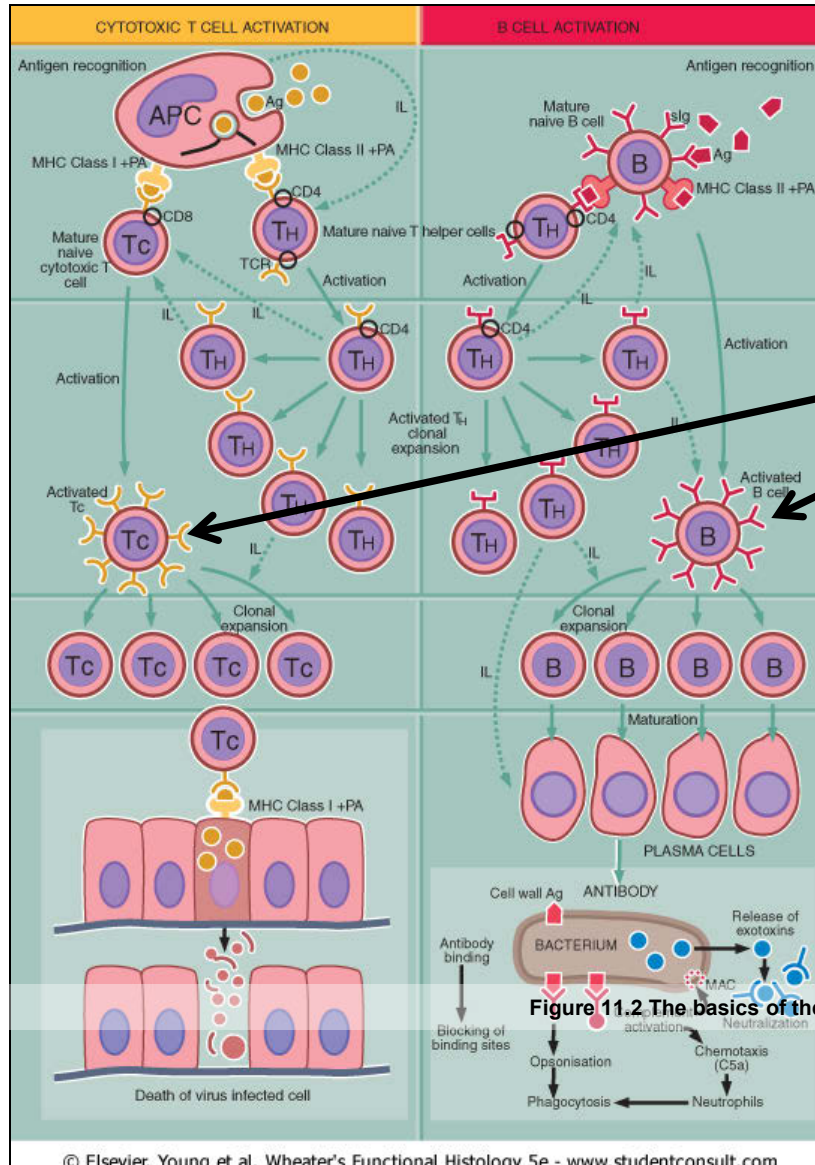


Figure 10-1 Lineage origin of the lymphoid progeny within the context of hematopoiesis

Immune Response



Immune Response



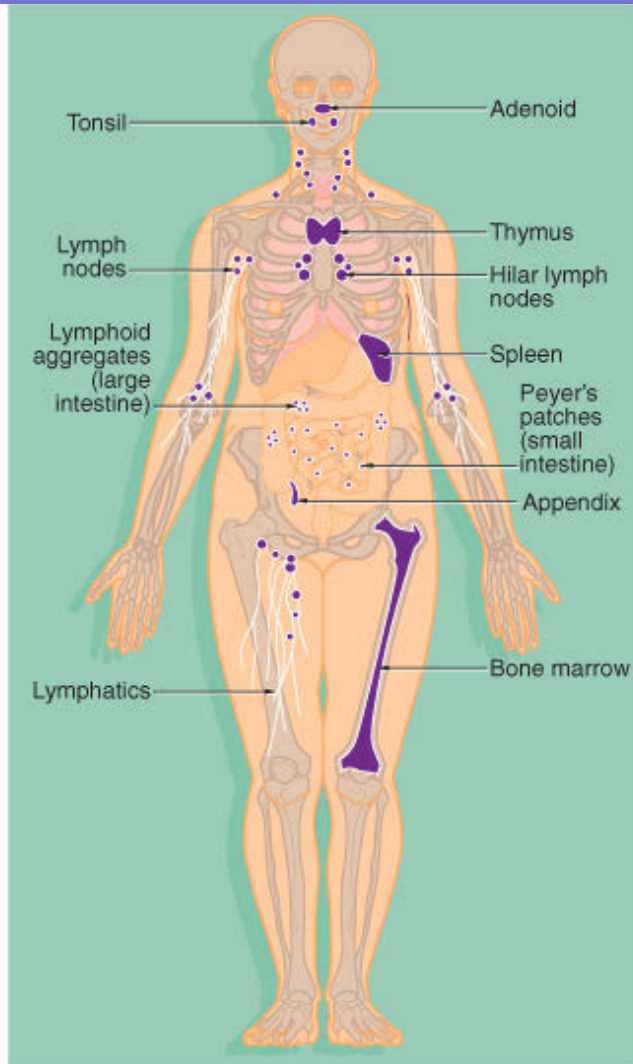
Persist as immunological memory cells for decades – measured at over 70 years!

Figure 11.2 The basics of the immune response

Brief History of Cell Biology and Polio Vaccine

- 1665 Robert Hooke – first microscope; saw cells named them
- 1839 Cell Theory – the cell as a unit of biology; cells as building blocks of tissues and organs; cell division
- 1860 and later – Pasteur and others showed that disease, etc. come from germs (not spontaneously) → lead to sterile surgery in 1870's by Lister
- 1890's viruses discovered – using filters smaller than bacteria!
- 1906 Harrison invents tissue culture (eggs as medium)
- 1931 viruses grown in hen's eggs
- 1948 Enders, Weller and Robbins grow polio virus in human fetal kidney cells (Nobel Prize, 1954)
- 1952/3 58,000/35,000 polio cases in US
- 1950's Salk and Sabin (and others) develop vaccine for polio
 - Accompanied by major advances in tissue culture methods
 - Funding first from March of Dimes; then from newly formed NIH
 - Killed virus vs attenuated virus → inactivated virus (current vaccine)
 - Now know that Sabin strain (oral vaccine) differs in 57 nucleotides – these changes contain virus in the gut (out of nervous tissue) -> allow antibodies to form
 - Polio virus is RNA virus with only 7500 nucleotides!
- 1961 161 cases in US!
- Polio declared eradicated in US in 1994; China and Australia in 2000; Europe in 2002. (Remains endemic in parts of Africa and Asia → continued need for vaccination.)

The Immune System



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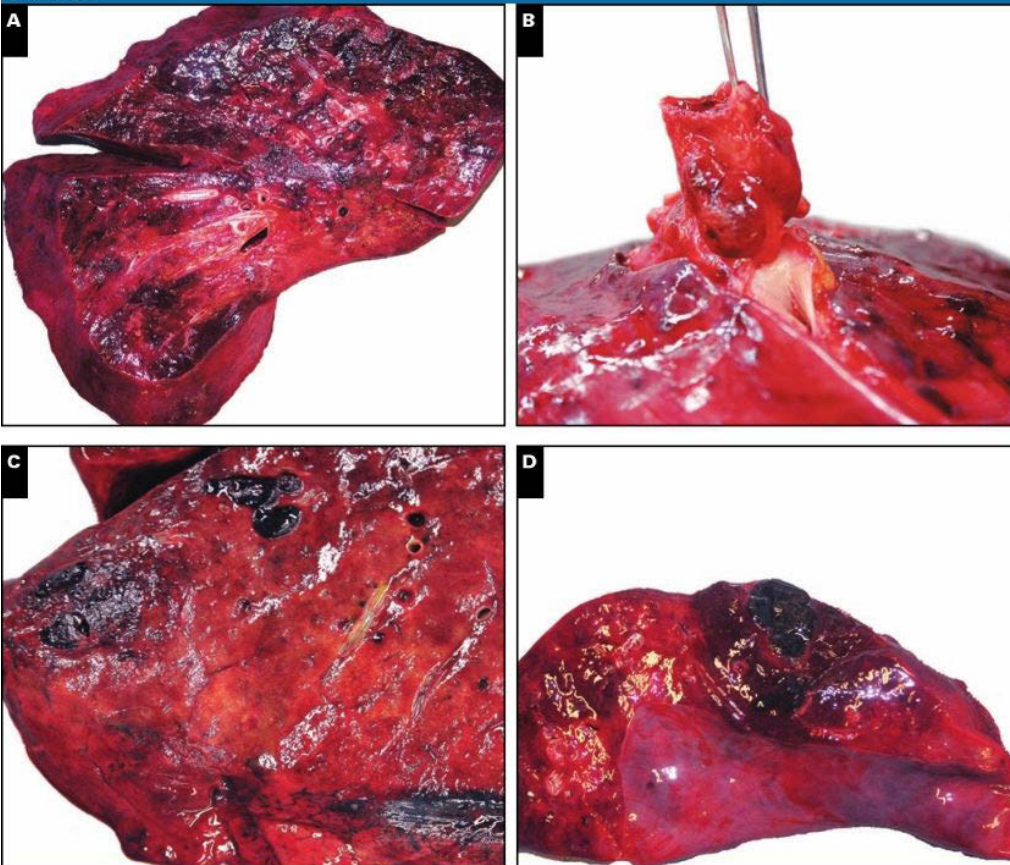
Figure 11.1 The organs of the immune system; Ag antigen APC antigen presenting cell B B lymphocyte IL interleukin MAC membrane attack complex MHC major histocompatibility complex PA processed antigen slg surface immunoglobulin Tc cytotoxic T cell TCR T cell receptor Th T helper cell

Polio Virus vs Flu Virus

- **Polio virus is an RNA virus**
 - only 7500 nucleotides
 - one gene and one protein
 - Single host (HUMAN)
 - considered to be one of the simplest viruses
- **Flu virus is also an RNA virus**
 - 13,588 nucleotides
 - 8 strands of RNA (8 genes)
 - Allows recombining
 - 11 proteins
 - Multiple hosts in many species (human, pigs, birds, etc.)
 - High mutation rate → rapidly changes

Randall

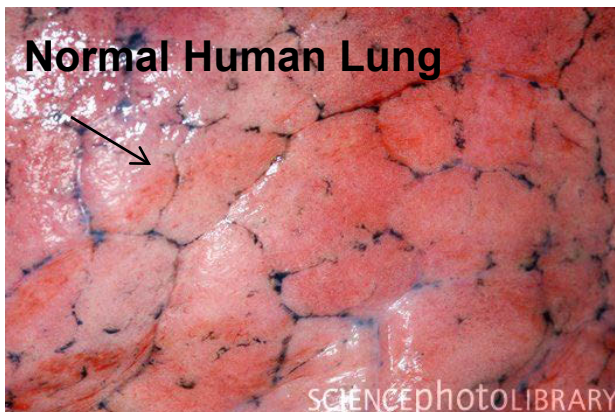
- 50 year old avid runner
- 2009 he became severely ill with fever to 102, fatigue, severe body aches and dry cough and sore throat
- Two days later collapsed at neighbor's
- Taken to Emergency room, admitted to Intensive Care
- Placed on ventilator (respirator)
- Developed coma, kidney failure, died 5 days later



Source: Am J Clin Pathol © 2010 American Society for Clinical Pathology

Post Mortem (autopsy) Findings:

- Lungs weighed two and a half times expected
- Tissue was heavy, dense and filled with fluid and blood
- The extensive tissue damage to the air sacs explained inability of his lungs to be ventilated
- Cultures of lung tissue Positive for Influenza virus



Influenza symptoms

- **FEVERS (92%)**
- **COUGH (72%)**
- **RUNNY NOSE (62%)**
- **SORE THROAT (28%)**
- **HEADACHE (22%)**
- **FATIGUE (10-15%)**
- **BODY ACHES (8%)**



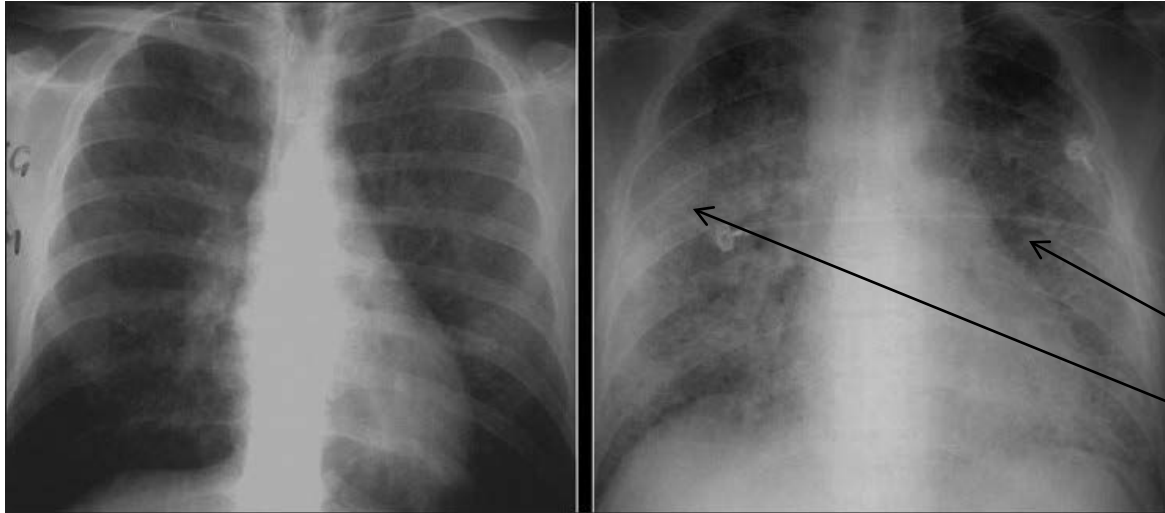
INFLUENZA: COMPLICATIONS

(In order of severity)

- Death
- Multiorgan System Failure (lungs, kidneys, brain swelling, etc)
- Pneumonia (primary viral), secondary (bacterial)
- Flare-up of underlying lung, heart diseases
- Guillain Barre Syndrome (ascending paralysis), Seizures (children), Reye syndrome

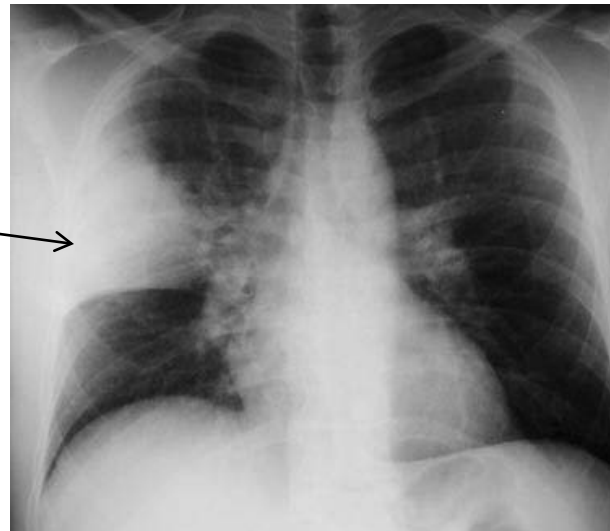
Influenza: lung complications

Normal



**“Primary”
Influenza
pneumonia**

**Secondary
Bacterial
pneumonia**



Those at higher risk for complications...

- Younger children
- Older Adults (>65)
- Pregnancy
- People with chronic medical illnesses (diabetes, congestive heart failure, kidney disease, emphysema/COPD, etc)
- Those with weakened immune system (HIV, cancer/leukemia, transplant patients)

**1918: 40% morbidity, many deaths (age 20-40)
40-50 million dead?**

1957: > 70,000 deaths in US

1968: > 30,000 deaths in US

**Every year, 100.000 hospitalizations related to
Influenza, about 30,000 deaths in US**



Influenza: Disease Burdens and costs 2003*

(Molinari, 2007: Vaccine Journal)

- 3.1 million hospitalized days, 31.4 million outpatient visits
- Direct Medical Costs: \$10.4 billion
- Projected lost earnings from illness and loss of life: \$16.3 billion
- Grand Total Bill: \$87.1 billion

*projection based on 2003 US
Population, probabilistic model



Influenza is a ZONOTIC disease

*Exists in **animals** (birds, pigs, etc)
and by transmission infect **human
animals***

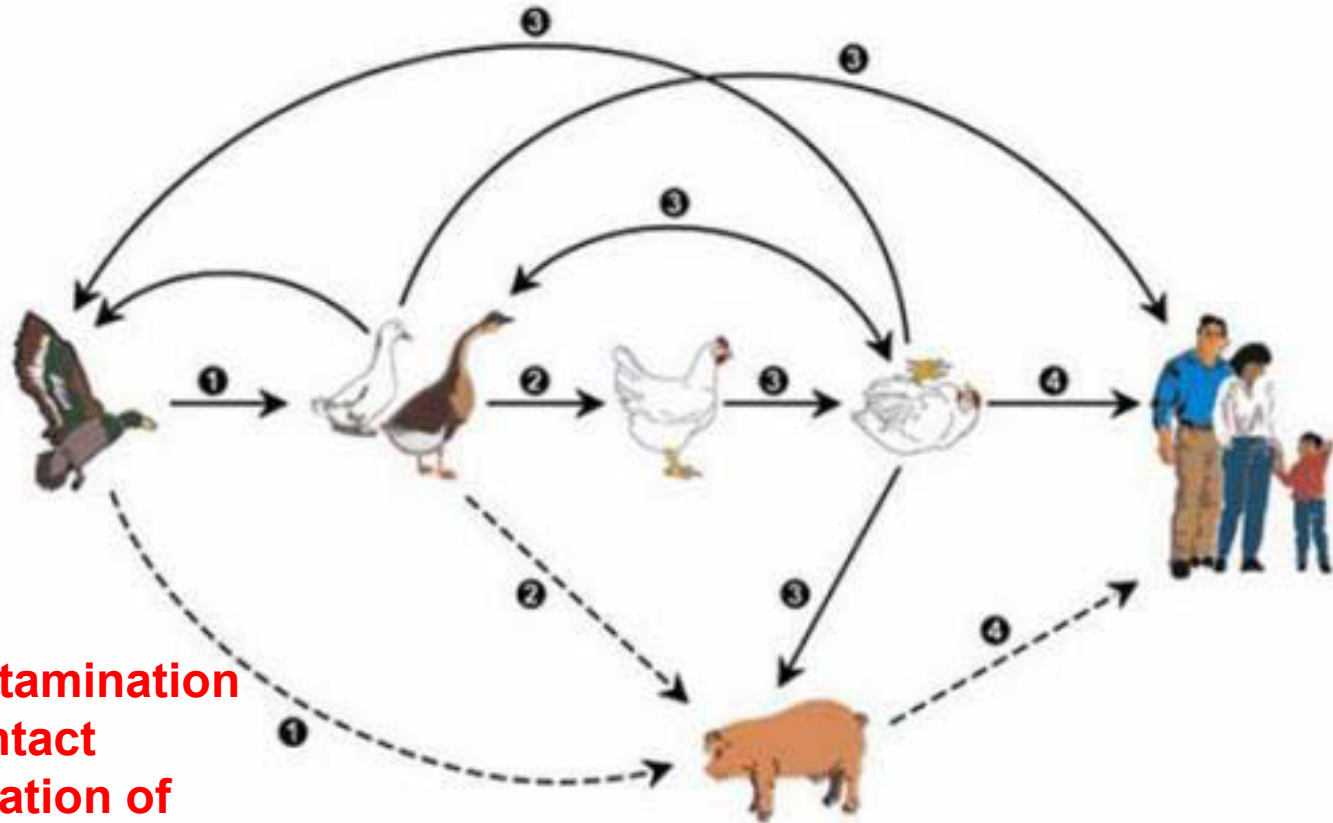
Influenza “A” (**A**wful)

Influenza “B” (less **B**ad)

Influenza “C”



Influenza A Ecology



- Fecal contamination
- Direct contact
- Contamination of water supplies

Source: <http://www.fda.gov>

What's in a name?

“H” = Hemagglutinin

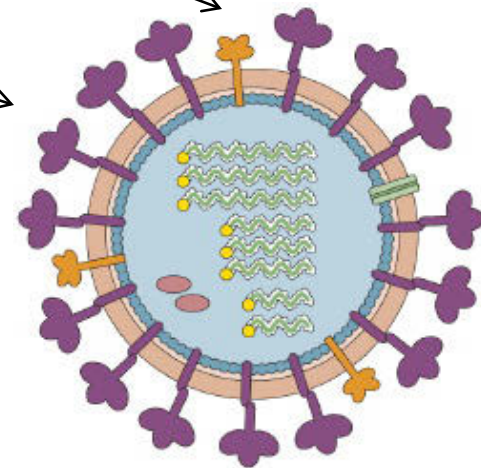
“N” = Neuraminidase virus coating proteins

H 1 N1 Spanish flu (1918)
Swine flu (2009)

H2 N 2 Asian flu (1957)

H3 N 2 Hong Kong (1968)

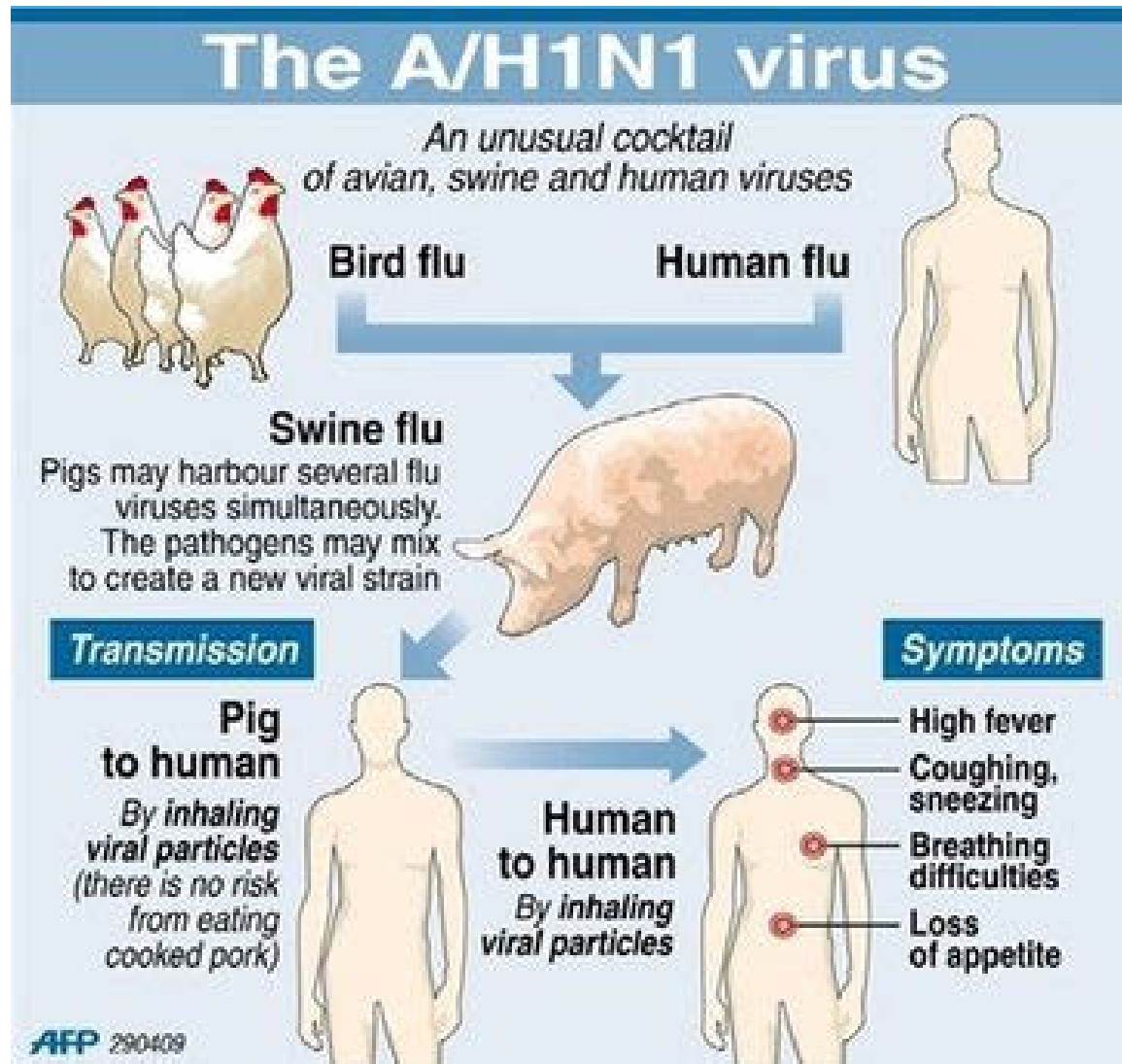
H5 N1 Bird flu (2004)



The Perfect Storm....

Frequent
Mutations
(genetic
change,
Mixing)

Influenza A
3x faster
Mutation
rate than B



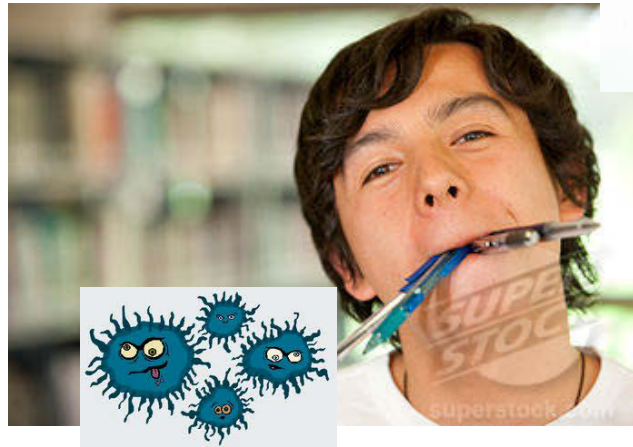
Interspecies
Infection,
“mixing”

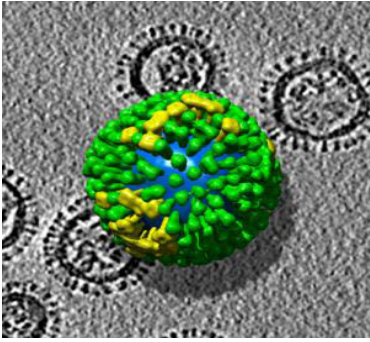
How you can “catch” viruses:

- Mostly by spread of floating aerosol droplets (influenza,, adenovirus, SARS, Hantavirus, enteroviruses, etc)
- Mucosal contact with hand/eye/nose (**fomites**)

What is a FOMITE????

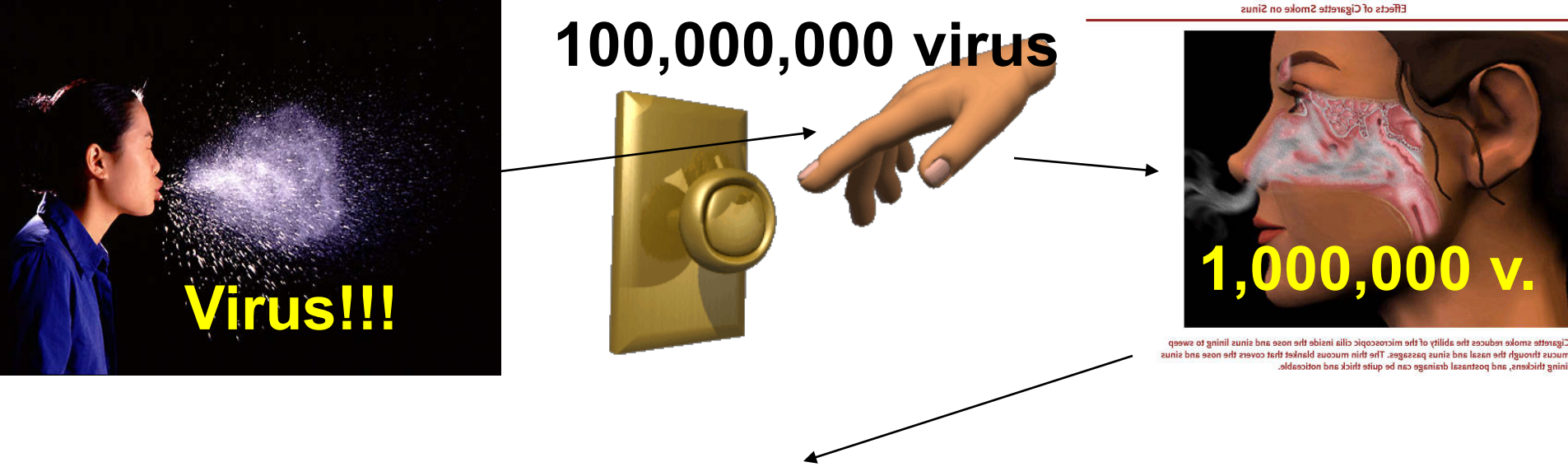
Fomite: inanimate object(s) capable of transmitting infectious organisms from one individual to another...





Did you know?

- Influenza virus is viable (survives) for 48hrs on metal or hard plastic smooth surfaces
- Viable for 12-24hrs on porous surfaces (paper, cloth)
- Viable on your skin for 5 min...
- Infected person is contagious about 24 hrs before onset of symptoms, through 5 days after...



Acid, “unfriendly” environment caused by normal bacteria (regular residents) on membranes of nose mouth and throat cause vast majority of viruses to be inactivated...but

Surviving virus enters epithelial (lining) cells in throat

Making a diagnosis:



- **Clinical symptoms in the right setting**
- **Nose/mouth swab (rapid turnaround)**
- **Virus Culture**

Prevention and Treatment Issues

- Antiviral drugs (two in clinical use); limited by efficacy, resistance and adverse effects, cost
- Symptomatic care (over counter symptom relief, hydration, etc)
- Prevention is cornerstone of control

Surgical masks have been shown to protect HCW's from SARS...



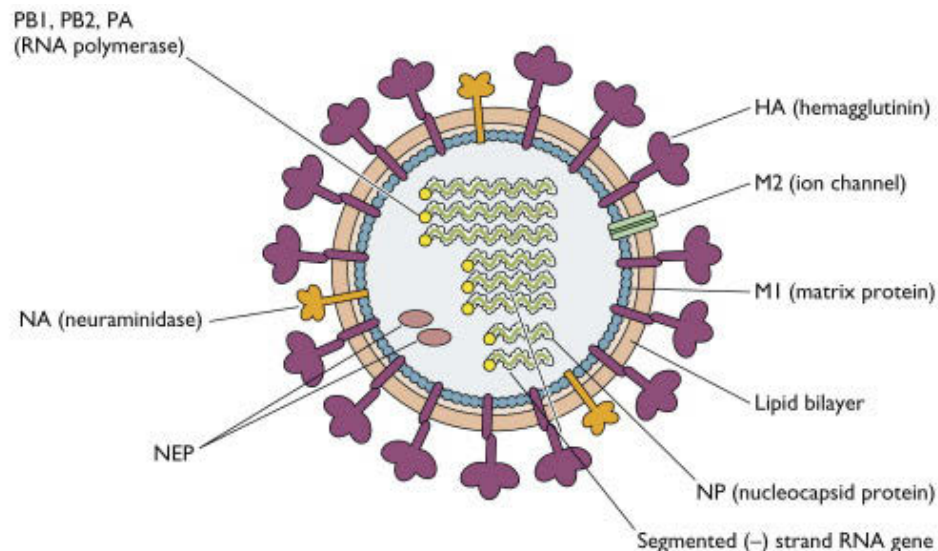
Japanese routinely wear masks when sick...

Prevention and treatment issues:

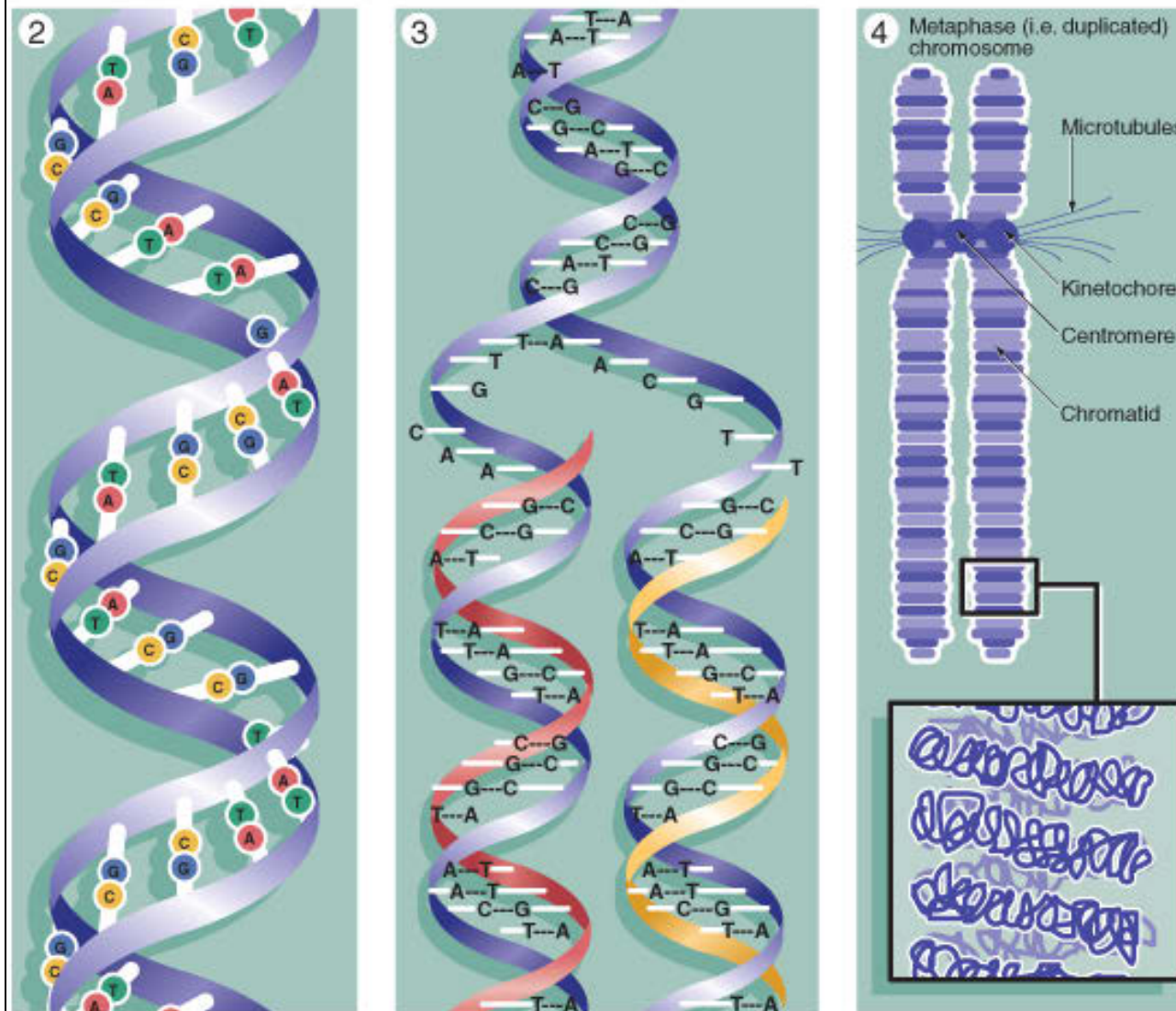


Flu Virus

- **Flu virus is also RNA virus**
 - **13,588 nucleotides**
 - **8 strands of RNA (8 genes)**
 - **Allows recombining**
 - **11 proteins**
 - **High mutation rate → rapidly changes**



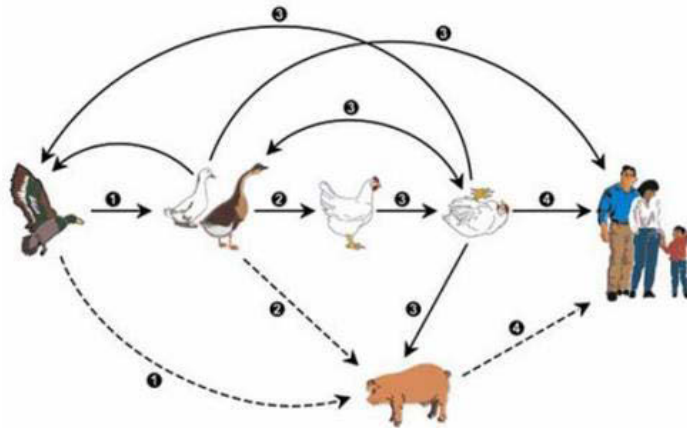
DNA is the Alphabet of Life (A, C, T, G)



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Figure 2.2 Chromosomes during mitosis; A adenine C cytosine G guanine P phosphate S deoxyribose T thymine

Influenza A Ecology



Source: <http://www.fda.gov>

28

(c) 2007, Joann M. Lindenmayer, D.V.M., M.P.H.

The genetic change that enables a flu strain to jump from one animal species to another, including humans, is called "ANTIGENIC SHIFT." Antigenic shift can happen in three ways:

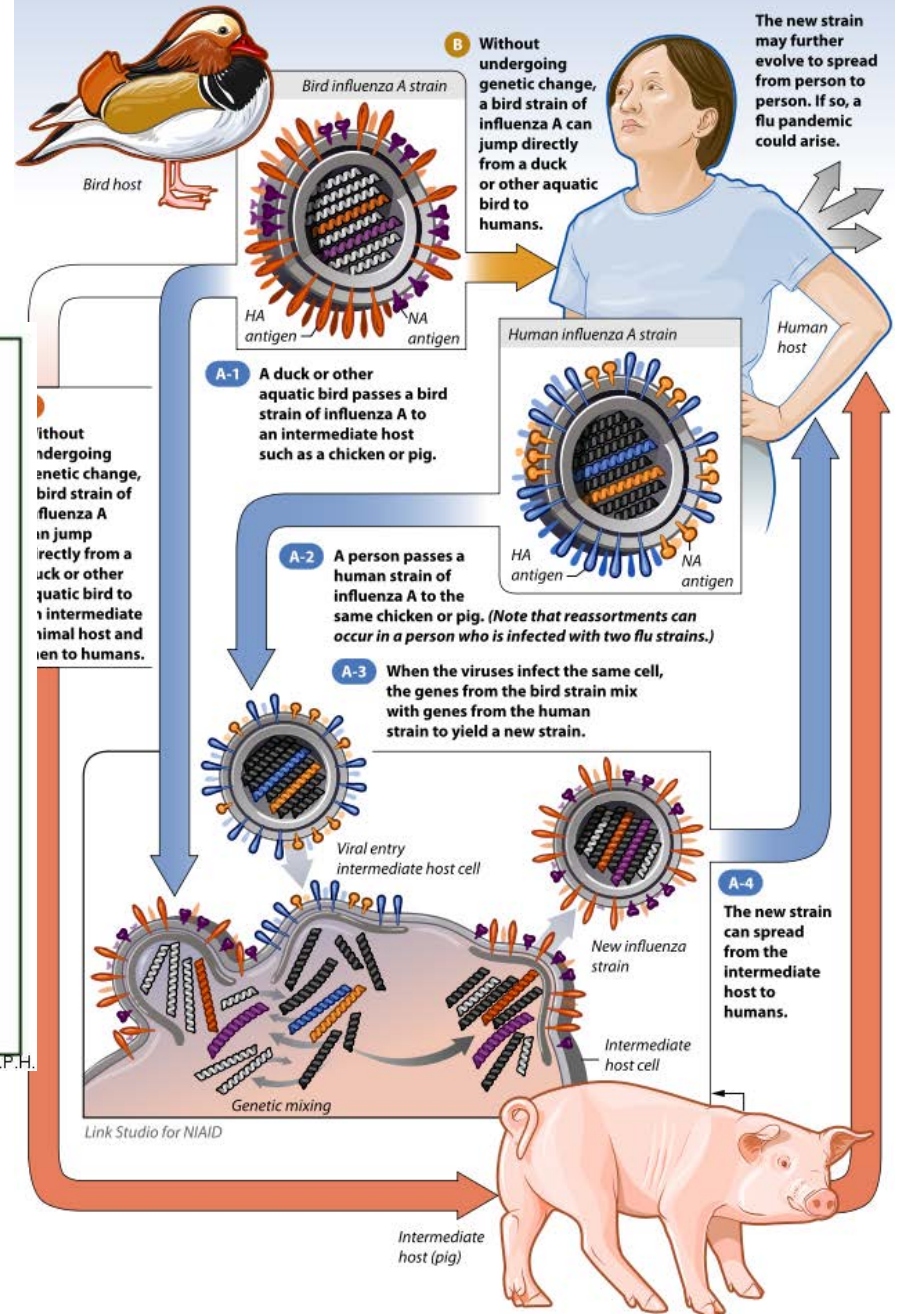
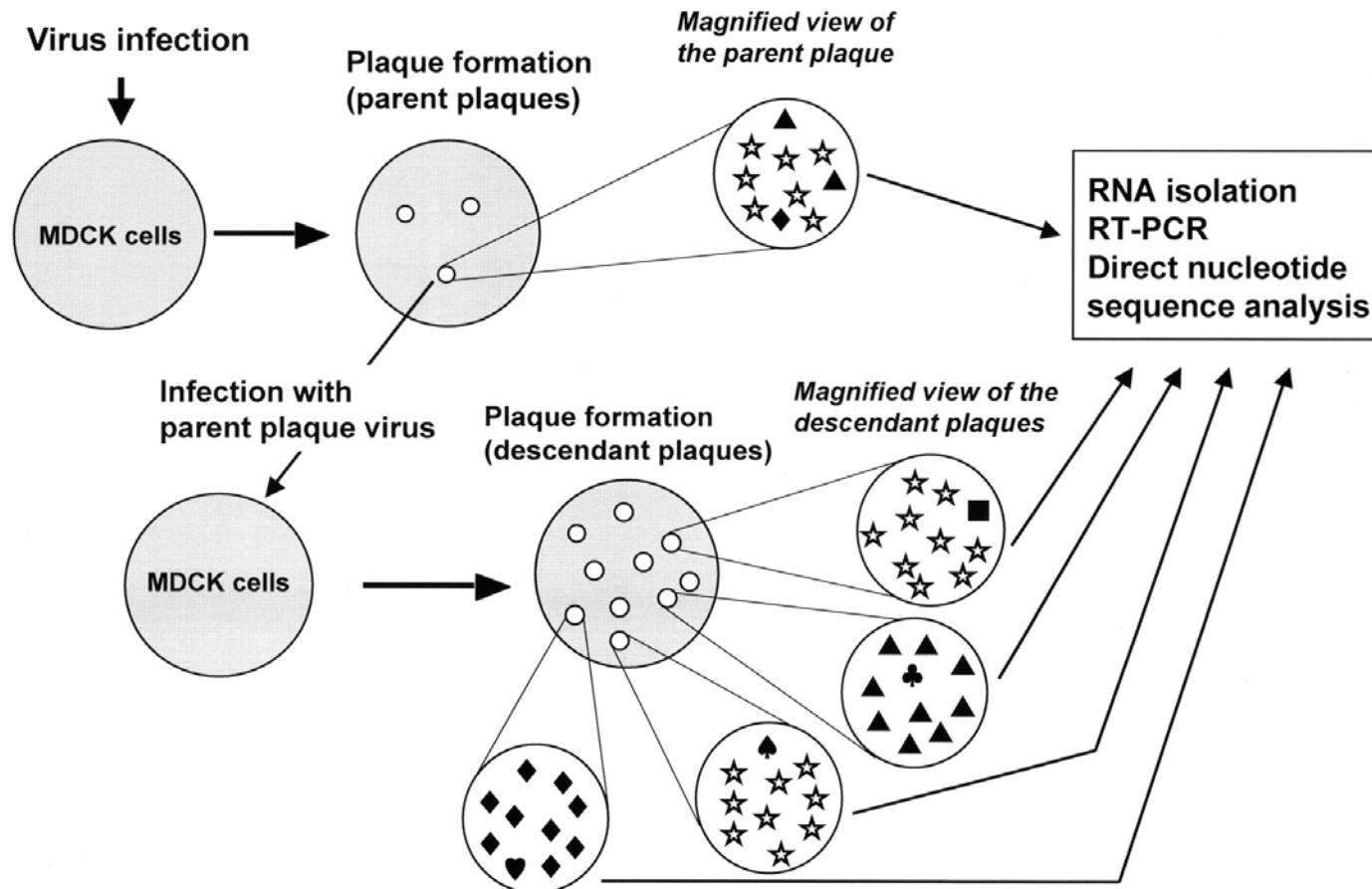
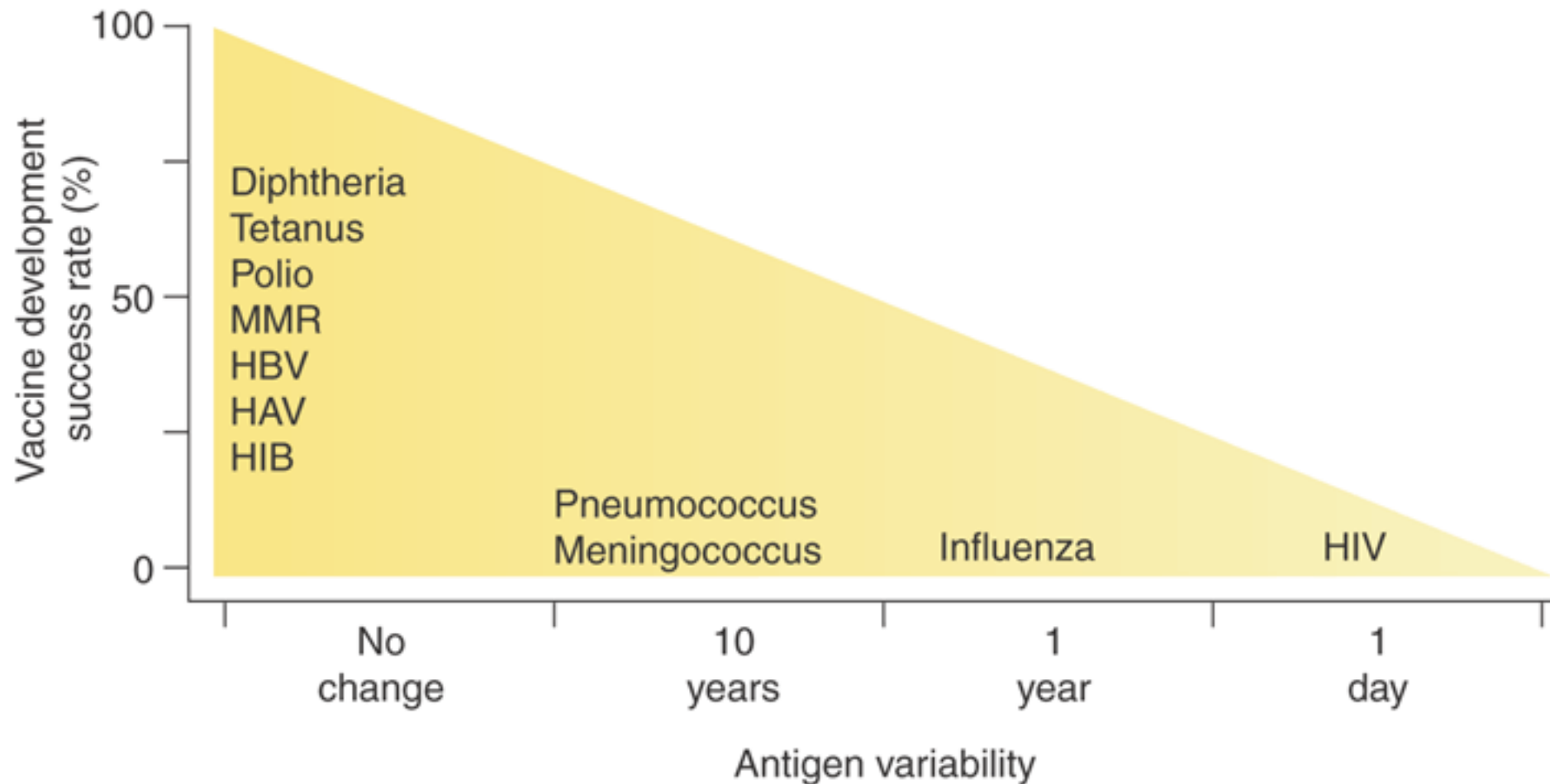


FIG. 1. Measuring the mutation rate during the growth of a single plaque



Nobusawa, E. et al. 2006. J. Virol. 80(7):3675-3678

The Complexity Increase Vaccine Development Success Rate!



DNA Sequencing to the Rescue!



...ACTCGTA...
...ACTCGTA...
...ACTCGTA...
...ACT**G**GTA...
...ACTCGTA...
...ACTCGTA...
...ACTCGTA...
...ACTCGTA...
...ACTCG**C**A...
...ACTCGTA...

CDC collects patient samples, identifies mutations as they occur → leads to changes design of new flu vaccine every year!

DNA Sequence Used to Make New Antigens for Vaccines

Sabin, Jenner

Salk vaccine

Killed
vaccines
& VLPs

Live attenuated
vaccines
& vectors

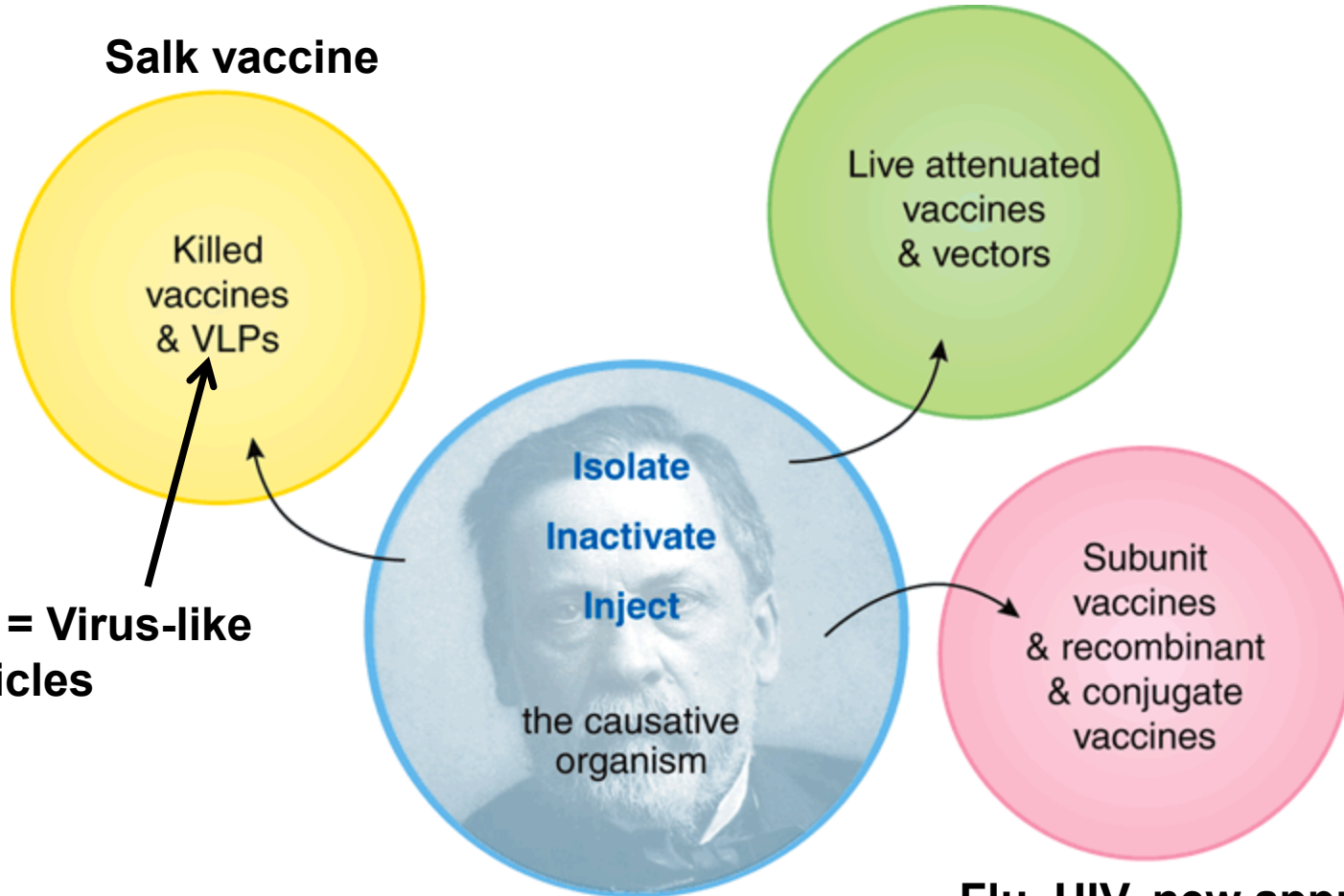
Isolate
Inactivate
Inject

the causative
organism

Subunit
vaccines
& recombinant
& conjugate
vaccines

VLP = Virus-like
particles

Flu, HIV, new approaches



Upcoming “Marriage Series”

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