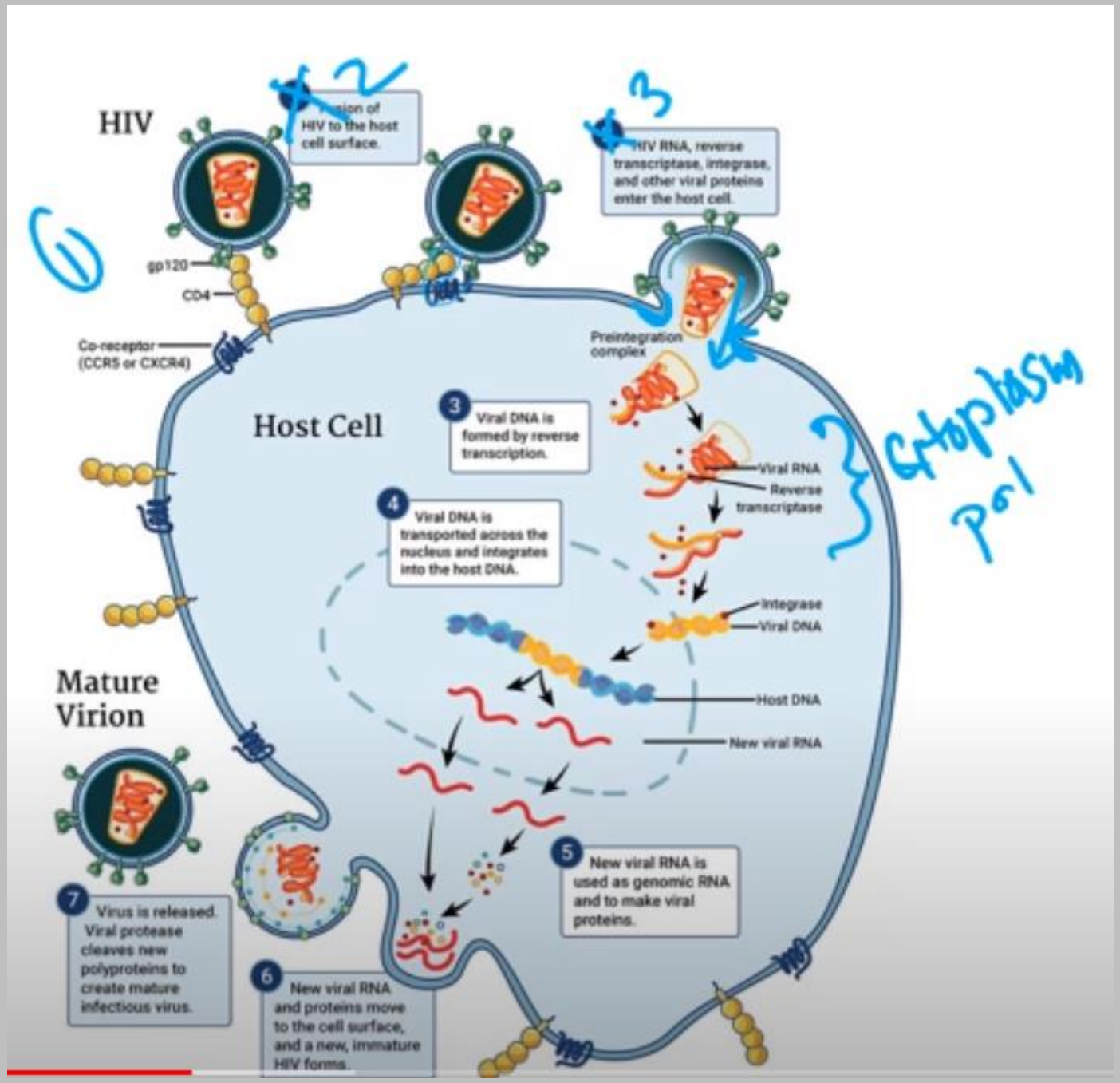
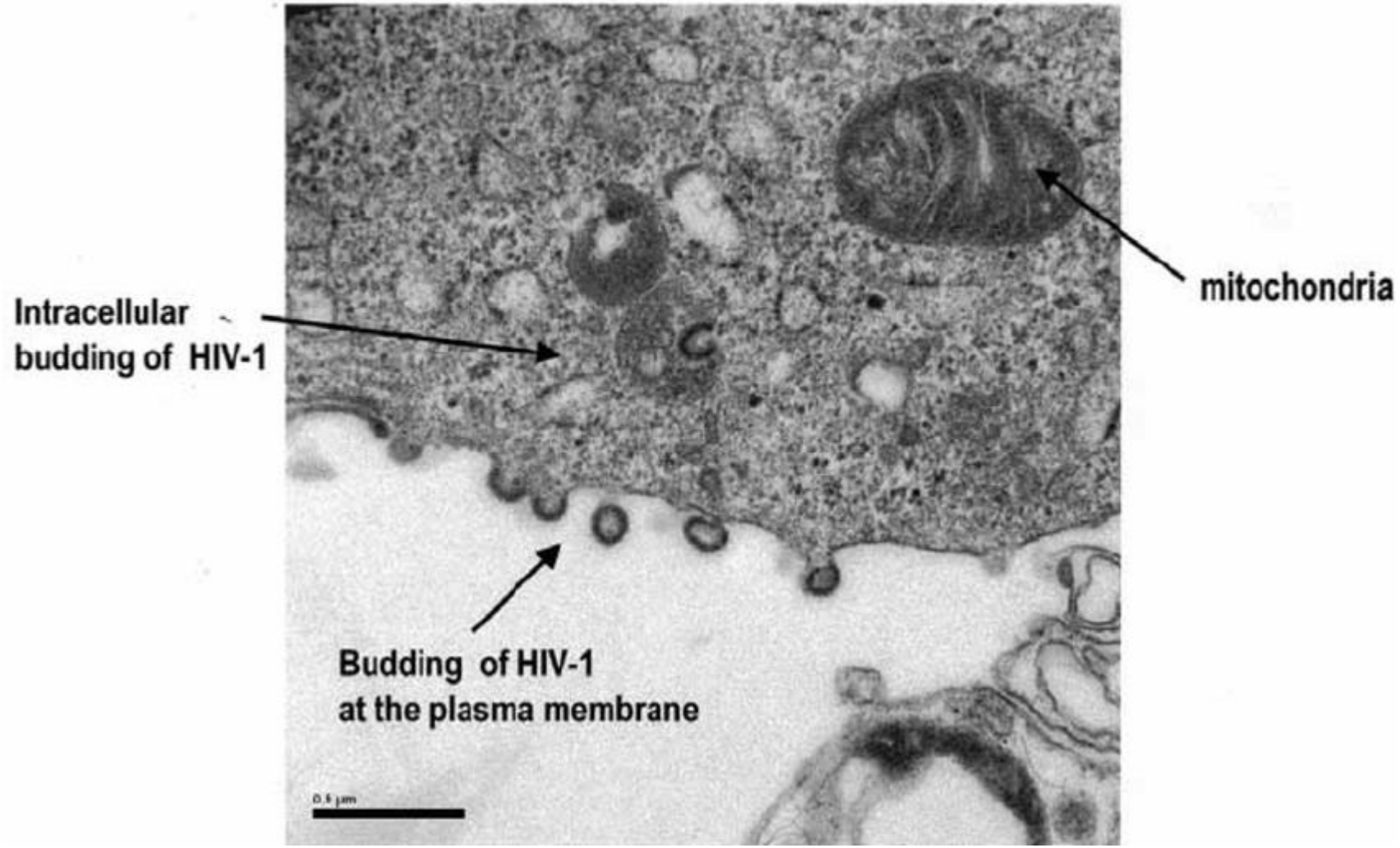
VISUALISATION OF INFECTION and IMMUNE CELL ACTIVITY

J. Skopalík
7.12.2021

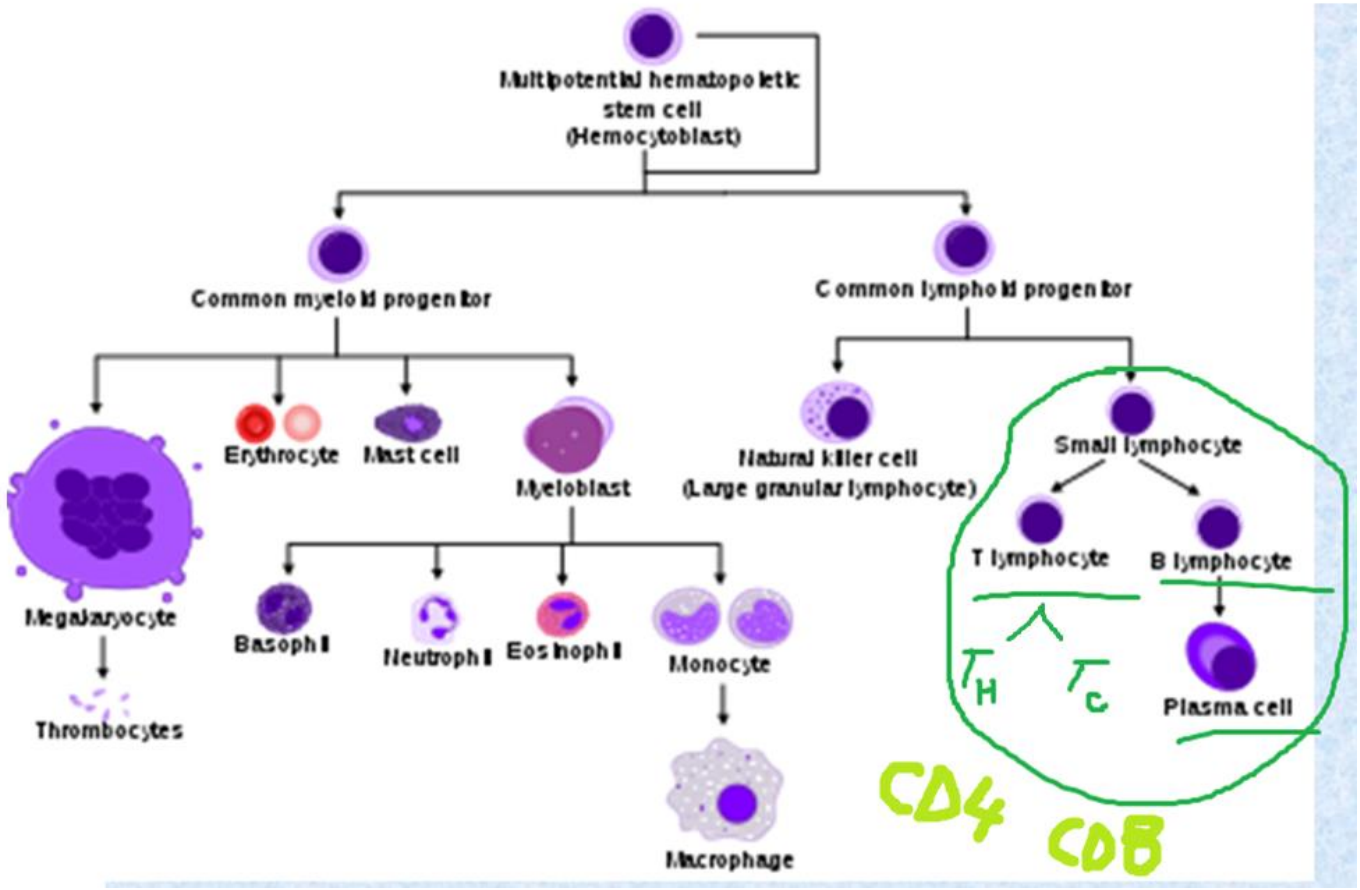
Typical scheme of VIRUS attack and replication inthe human cell:



Virus HIV – visualization by electron microscopy

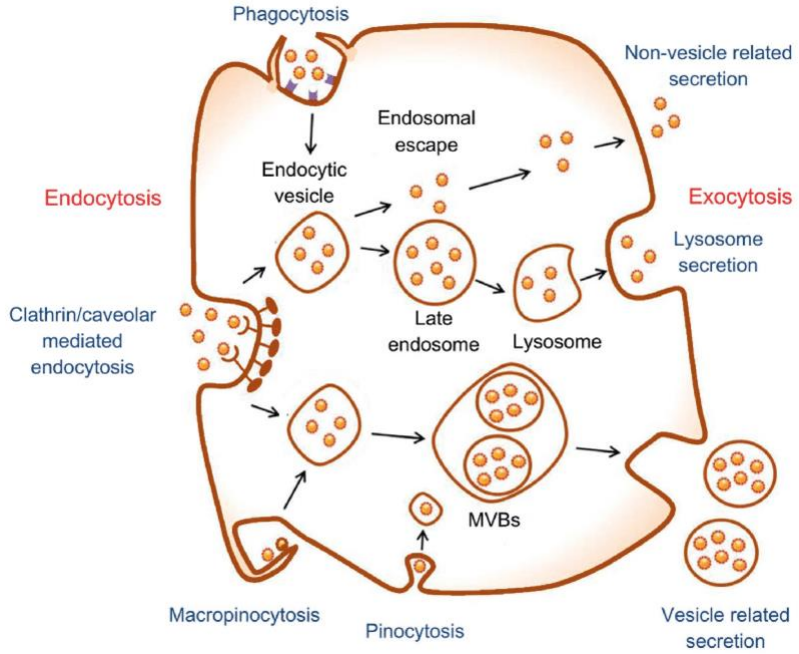


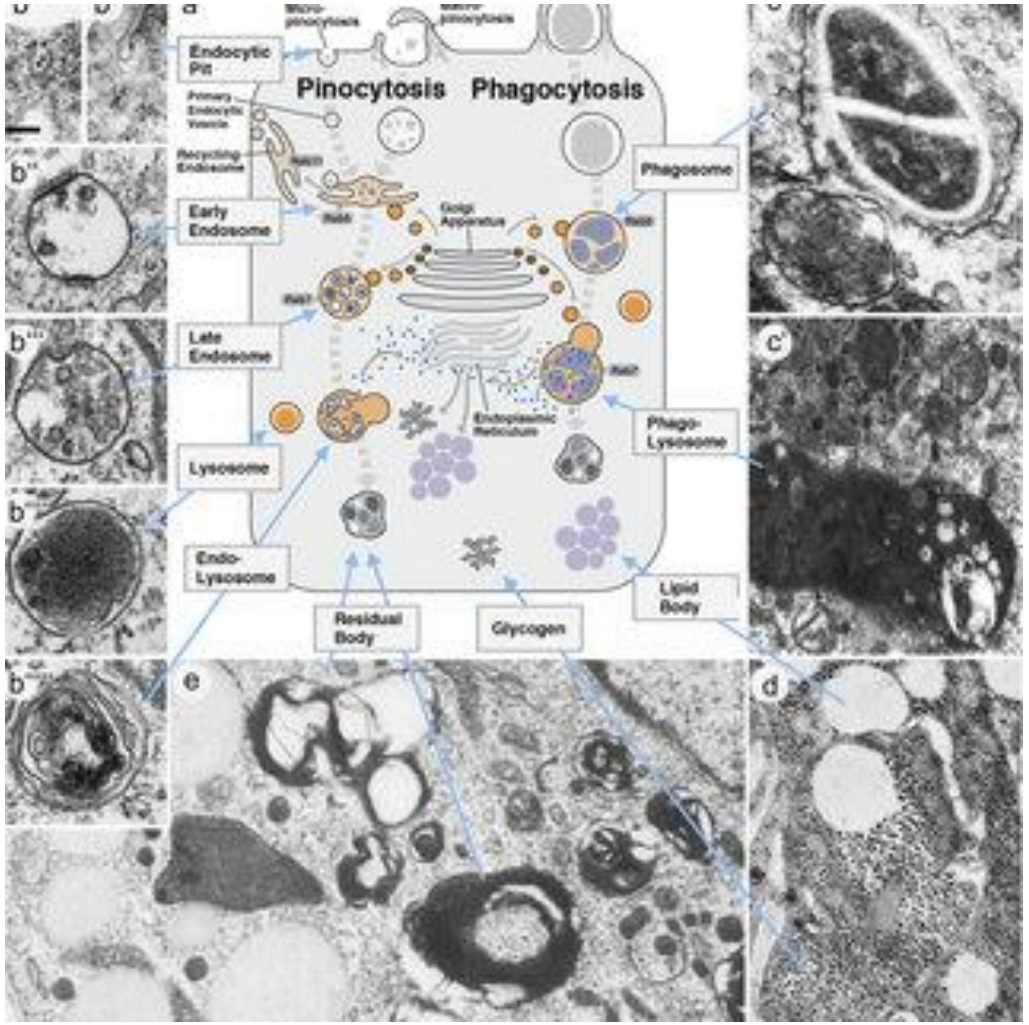
Remeber: in last lesson, we had some principial theory of immune cell system



In next pages, there will be focusing and methods for micro-**view** to Macrophage and Lymphocytes action

Bacteria capture and lysis in lysosome - visualization by electron microscopy



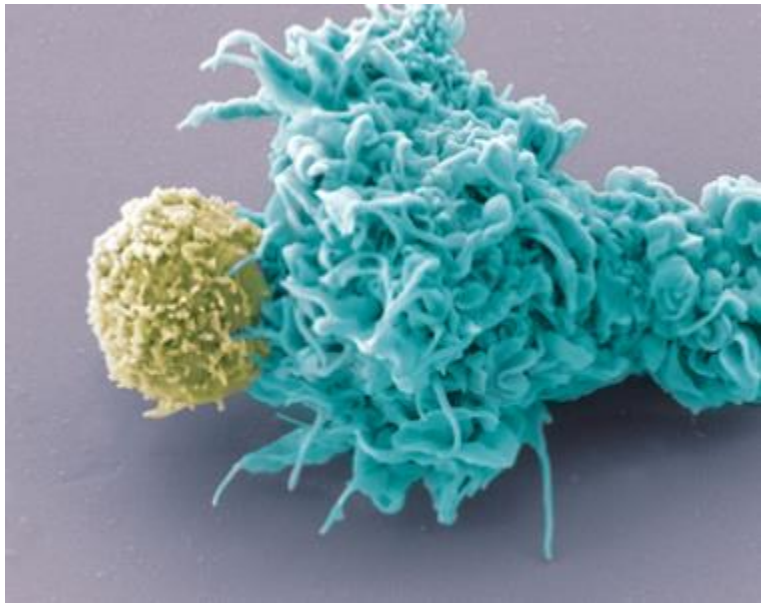


How to make visualisation of IMMUNE CELL

and <u>their INTERACTION</u> with PATOGEN or anoter CELL ????

First method: microscopy (good for science but not for hospital daily analysis)

\macrophage bacterium c4d



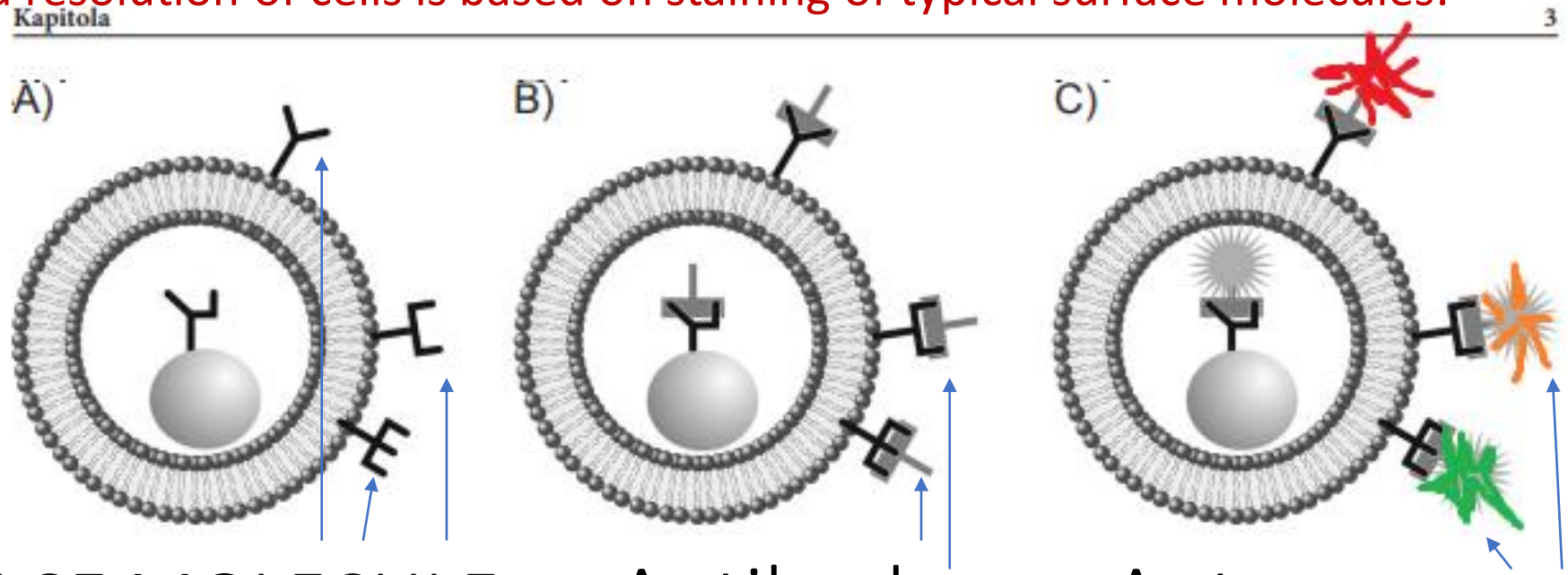
T lymph. / dendritic cell

Second method: flow-cytometry (good also for hospital daily analysis)

Physician take the blood sample or bone marrow bioptic sample, the cyometry is used for example to analysis of ratio **CD20 cells** : CD8 cells

or analysis of decreasing of CD8 lymphocyte and many other quantitative blood and immune cell analysis….

Staining and resolution of cells is based on staining of typical surface molecules:



SURFACE MOLECULE. Antibody Ant.+ Fluorescence Probe

typical surface molecules on T lymphocytes:

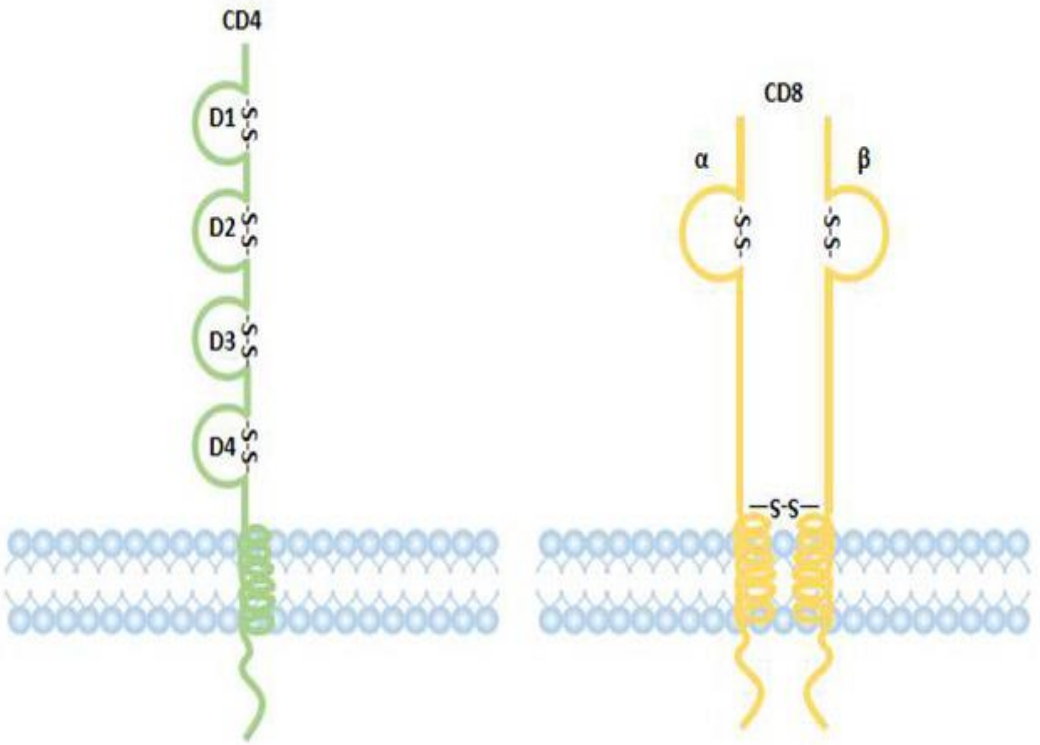
CD4

CD4 is a T helper cell marker, which is a single chain transmembrane protein. The extracellular structure belongs to IgSF, and there are four IgSF domains. The first and second domains can bind to MHC class II molecules. CD4 acts as a co-receptor for the TCR-CD3 complex recognition antigen and participates in signal transduction by binding to the MHC class II molecule, p56lek kinase.

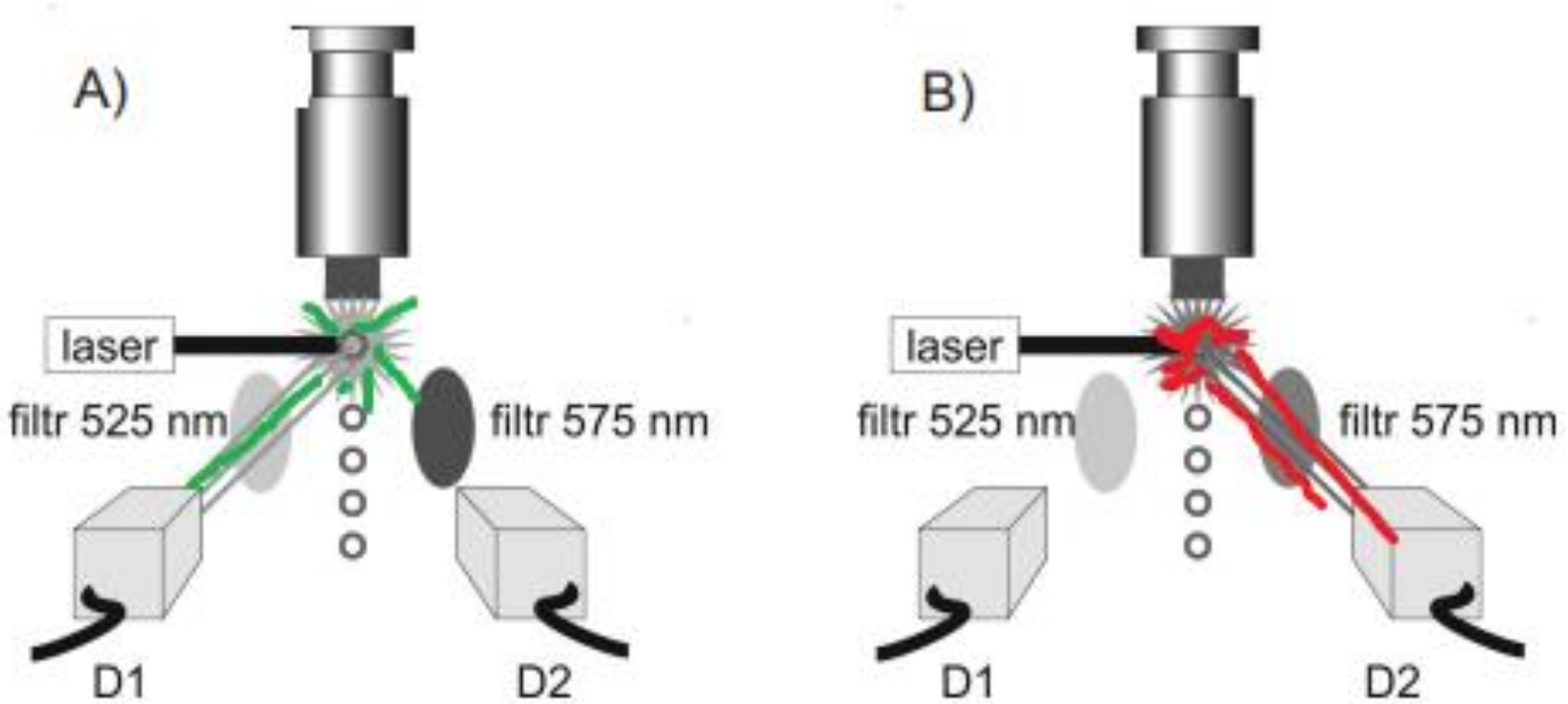
CD8

CD8 is a cytotoxic T cell marker, a heterodimer formed by the linkage of α and β chains by disulfide bonds, and the extracellular structure is an IgSF member. The cytoplasmic region of CD8 molecule can be combined with p56lek kinase to participate in signal transduction. CD8+T lymphocytes, which can specifically kill target cells, have anti-tumor, antiviral and important immunomodulatory effects, and their main function is to inhibit the immune response [15].

CD4 and CD8 molecules divide T cells into two distinct subpopulations. CD4 and CD8 are receptors of MHC class II or MHC class I molecules, respectively, and the changes in the number and ratio of CD4+ and CD8+ cells reflect the immune function status of the body.



The cells after stining by fluorescence antibody are collected in tube and droped ONE-AFTER-ANOTHER through the light of laser. After laser excitation, CD8 lymphocytes are mainly green fluorescence, CD4 lymphocyte are red fluorescence and detectors (D1 and D2 on figure) with computer compute the number of different type of cells in solution.



Obr. 3.14.2. Základní princip fluorescenčního modu průtokového cytometru – suspenze buněk je protlačována velmi ma-

VACCINES

J. Skopalík
7.12.2021

- Vaccines exploit the extraordinary ability of the highly evolved human immune system to respond to, and remember, encounters with pathogen antigens.

- A vaccine is a biological product that can be used to safely induce an immune response that confers protection against infection and/or disease on subsequent exposure to a pathogen. To achieve this, the vaccine must contain antigens that are either derived from the pathogen or antigen produced synthetically to represent components of the pathogen.

Statistical overview of post-vaccination elimination of diseases in Britain

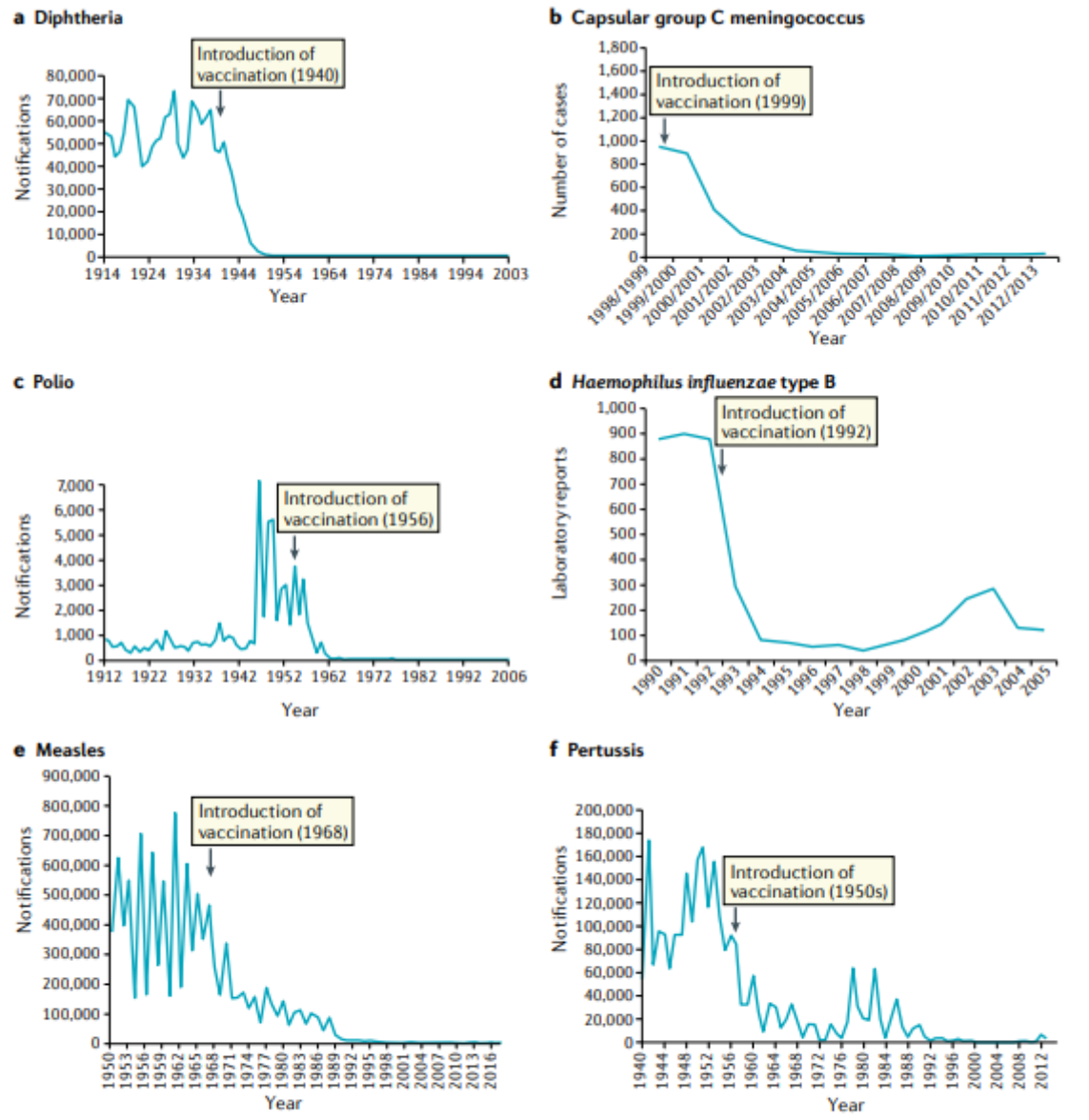
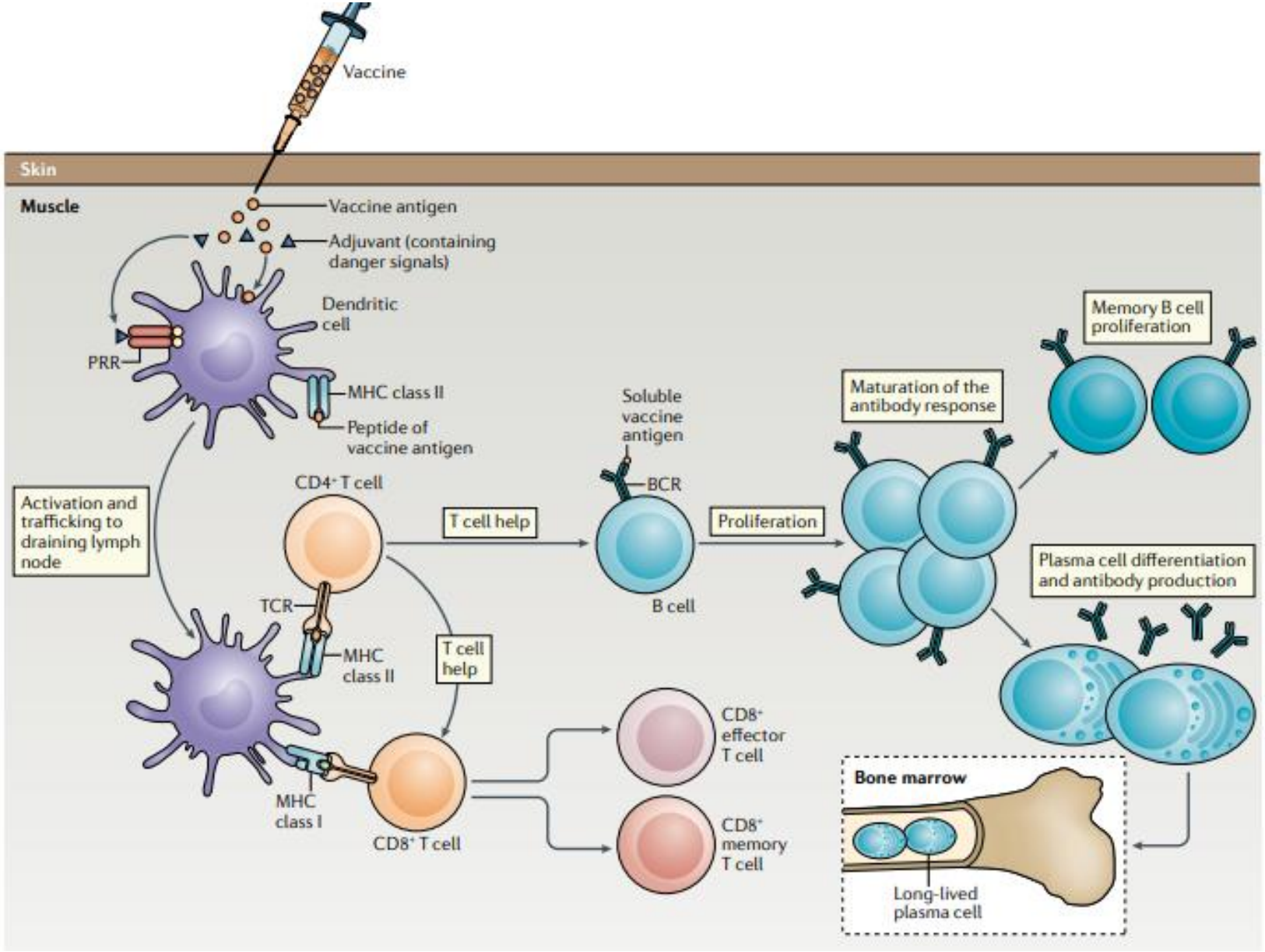
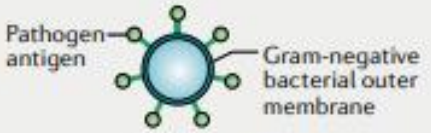
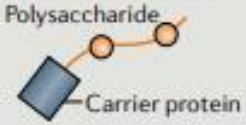


Fig. 1 | **The impact of vaccination on selected diseases in the UK.** The introduction of vaccination against infectious diseases such as diphtheria (part **a**), capsular group C meningococcus (part **b**), polio (part **c**), *Haemophilus influenzae* type B (part **d**), measles (part **e**) and pertussis (part **f**) led to a marked decrease in their incidence. Of note, the increase in reports of *H. influenzae* type B in 2001 led to a catch-up vaccination campaign, after which the incidence reduced. For pertussis, a decline in vaccine coverage led to an increase in cases in the late 1970s and 1980s, but disease incidence reduced

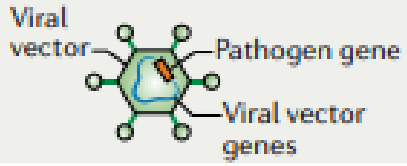
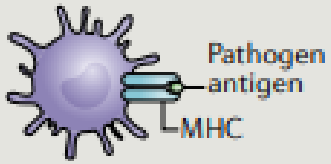
- The adaptive immune response is mediated by B cells that produce antibodies (humoral immunity) and by T cells (cellular immunity). All vaccines in routine use, except BCG (which is believed to induce T cell responses that prevent severe disease and innate immune responses that may inhibit infection; see later), are thought to mainly confer protection through the induction of antibodies (Fig. 3)



Traditional vaccines

Type of vaccine		Licensed vaccines using this technology	First introduced
Live attenuated (weakened or inactivated)		Measles, mumps, rubella, yellow fever, influenza, oral polio, typhoid, Japanese encephalitis, rotavirus, BCG, varicella zoster	1798 (smallpox)
Killed whole organism		Whole-cell pertussis, polio, influenza, Japanese encephalitis, hepatitis A, rabies	1896 (typhoid)
Toxoid		Diphtheria, tetanus	1923 (diphtheria)
Subunit (purified protein, recombinant protein, polysaccharide, peptide)		Pertussis, influenza, hepatitis B, meningococcal, pneumococcal, typhoid, hepatitis A	1970 (anthrax)
Virus-like particle		Human papillomavirus	1986 (hepatitis B)
Outer membrane vesicle		Group B meningococcal	1987 (group B meningococcal)
Protein–polysaccharide conjugate		*Haemophilus influenzae* type B, pneumococcal, meningococcal, typhoid	1987 (*H. influenzae* type b)

Modern vaccine

Type of vaccine		Licensed vaccines using this technology	First introduced
Viral vectored		Ebola	2019 (Ebola)
Nucleic acid vaccine		SARS-CoV-2	2020 (SARS-CoV-2)
Bacterial vectored		Experimental	–
Antigen-presenting cell		Experimental	–

- Detail literature source for advance study:

A guide to vaccinology: from basic principles to new developments
Andrew J. Pollard

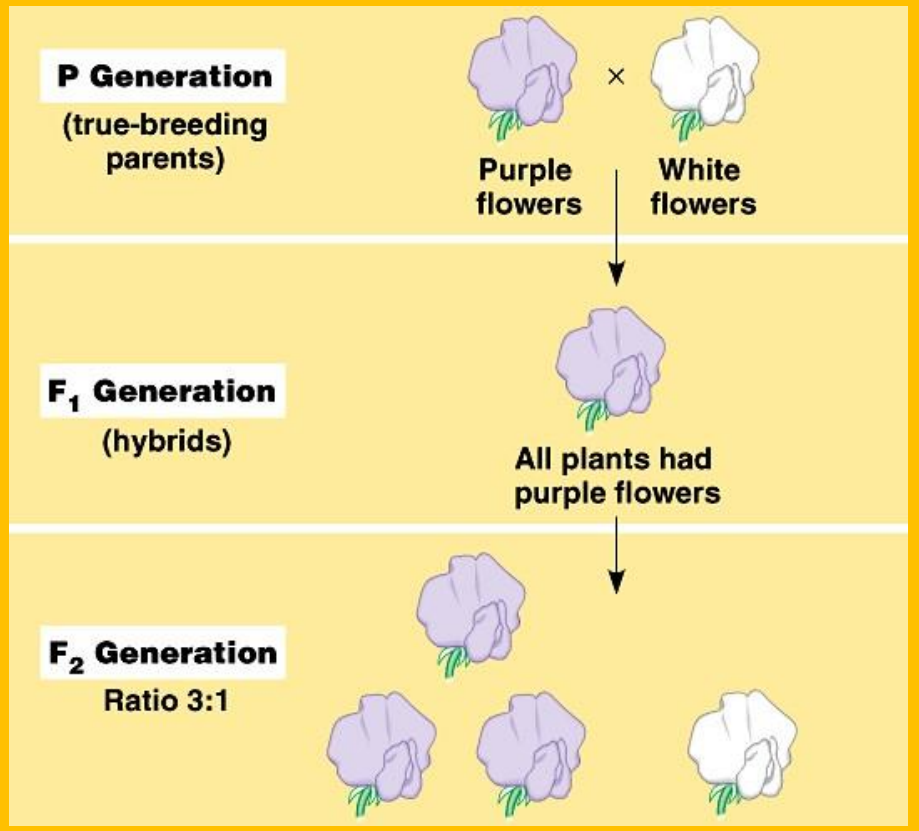
https://www.nature.com/articles/s41577-020-00479-7.pdf

Vaccination strategies - An overview

Giuseppe Del Giudice∗IRIS Research Center, Chiron SpA, Via Fiorentina 1,

BASIC CELL GENETICS

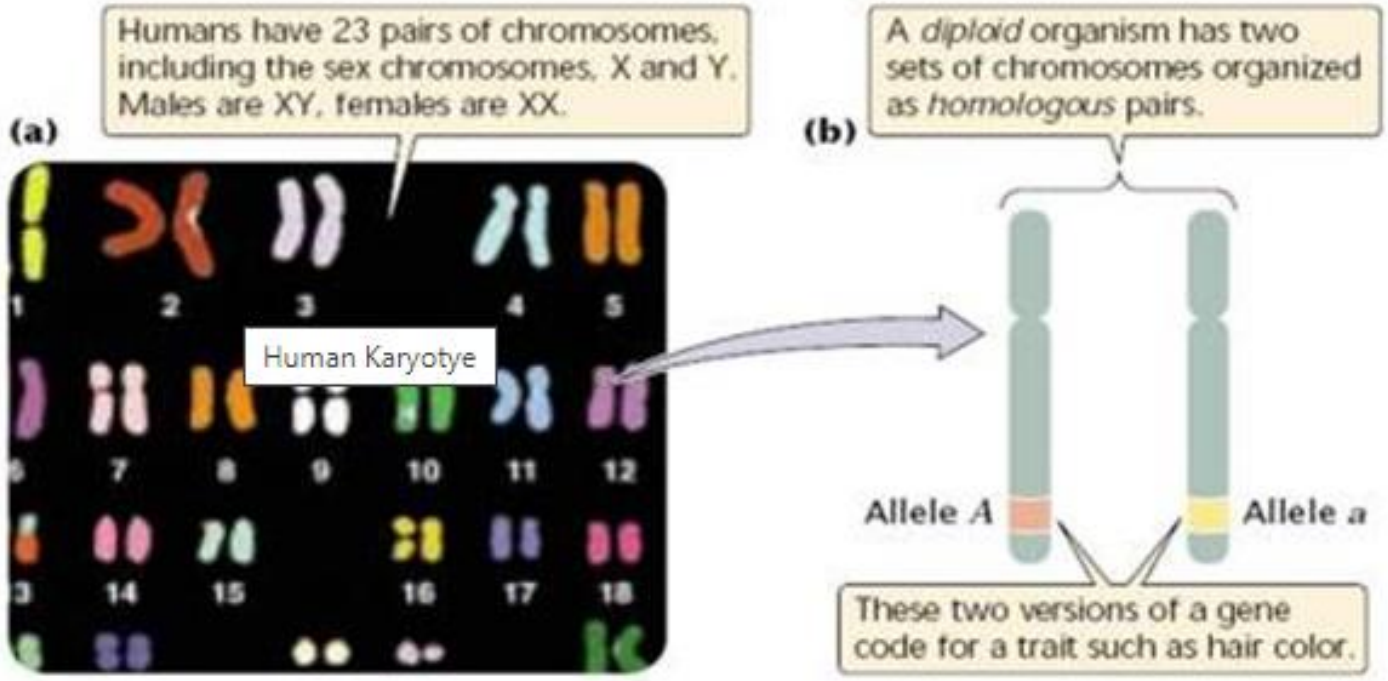
J.G. Mendel - mathematician, biologist, Augustinian friar and abbot of St. Thomas' Abbey in Brno



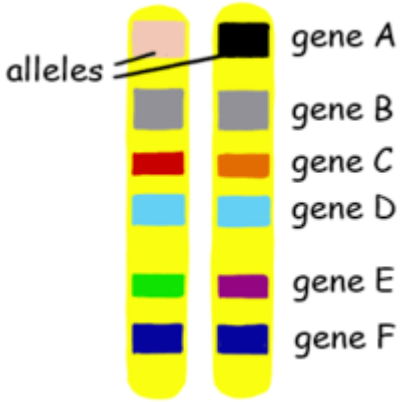
Mendel investigated why first generation F1 and second generation F2 of Pea Plant have unlogical color heredity

BASIC GENETIC FACTS

Actually a diploid organism has two copies of a gene or two sets of chromosomes, one from father's sperm and other from mother's egg. The chromosome of similar size and nature often form pairs during meiotic division and such identical chromosomes are called homologous chromosomes.



Human has many genes (color of eye, gene for hemoglobin structure, ...



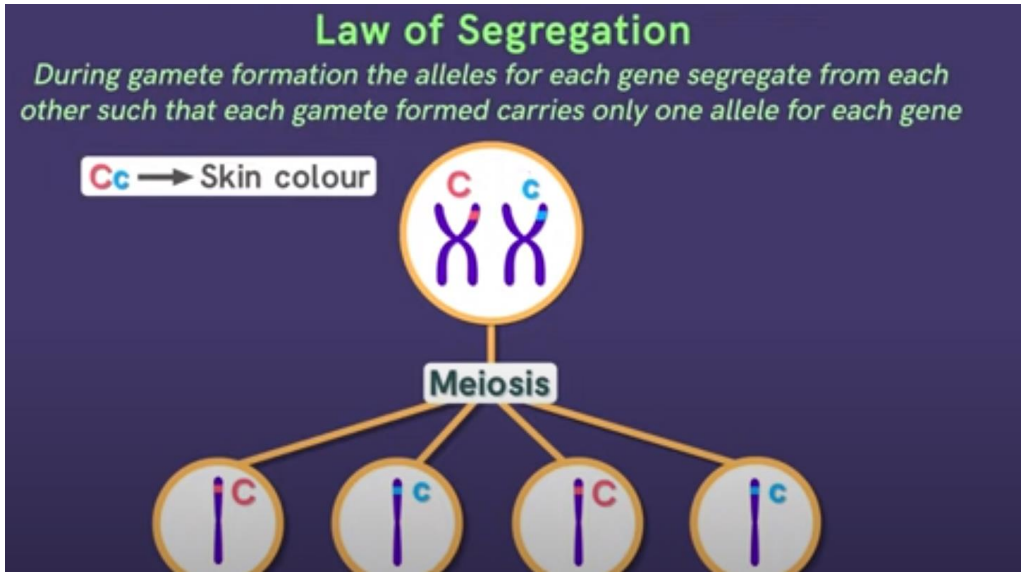
- **Mendel's basic conclusions for gene delivery** – MENDEL LAWs

1. **Law of Segregation:** When gametes form, alleles are separated so that each gamete carries only one allele for each gene
2. **Law of Independent Assortment:** The segregation of alleles for one gene occurs independently to that of any other gene*
3. **Law of Dominance:** Recessive alleles will be masked by dominant alleles†

* *The law of independent assortment does not hold true for genes located on the same chromosome (i.e. linked genes)*

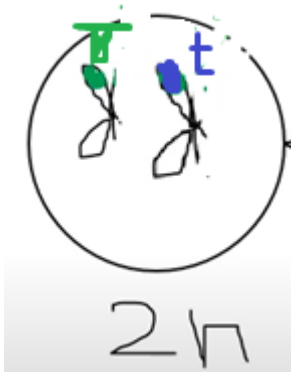
FIRST MENDEL LAW

- Simple, but not very good illustration :



FIRST MENDEL LAW

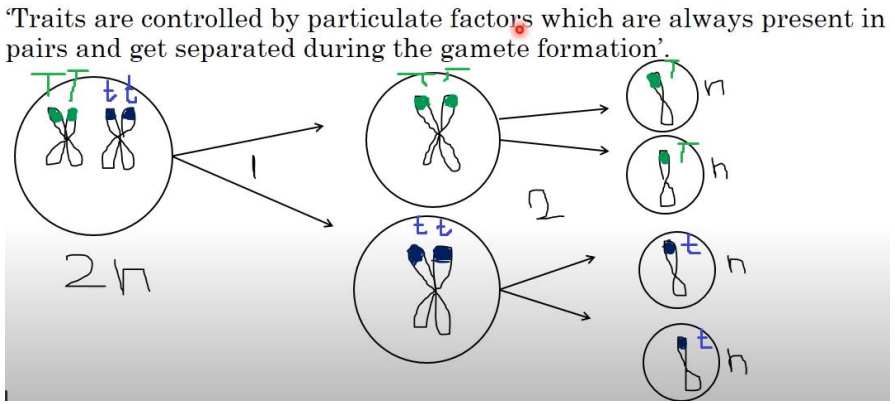
- Better ilustrations:



CELL before MEIOSIS

(T-gene in dominant form

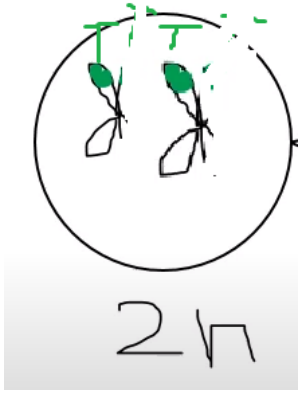
t -gen in recesive form)

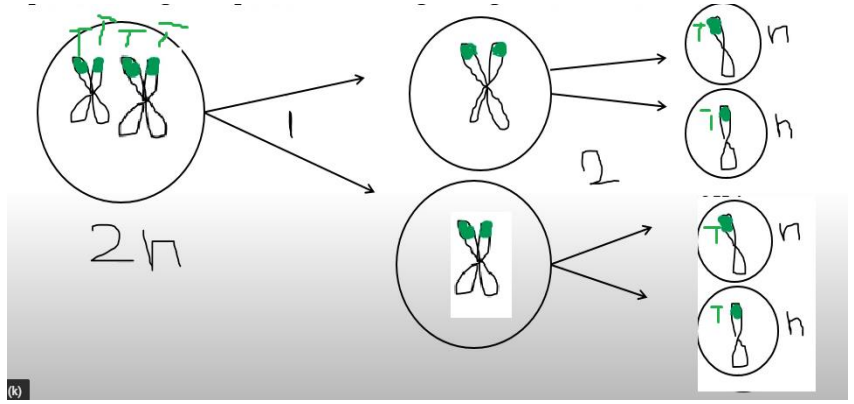


After chromoson duplication

After ANAPHASE I

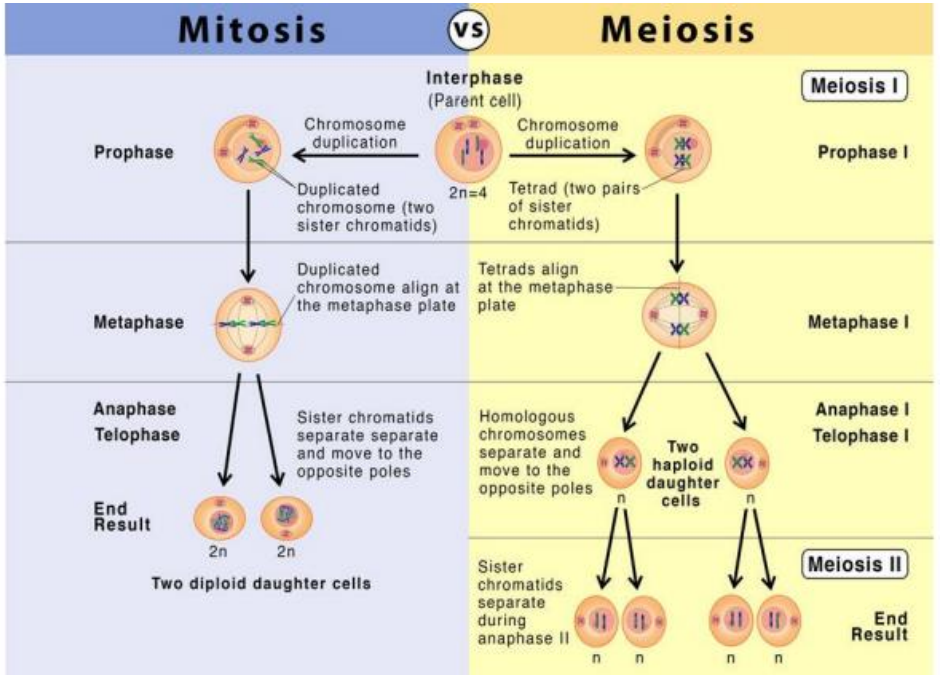
Delivery of different T or t into Gamets





Why are 4 alelles at final stage stage oriniated from one parentcell??

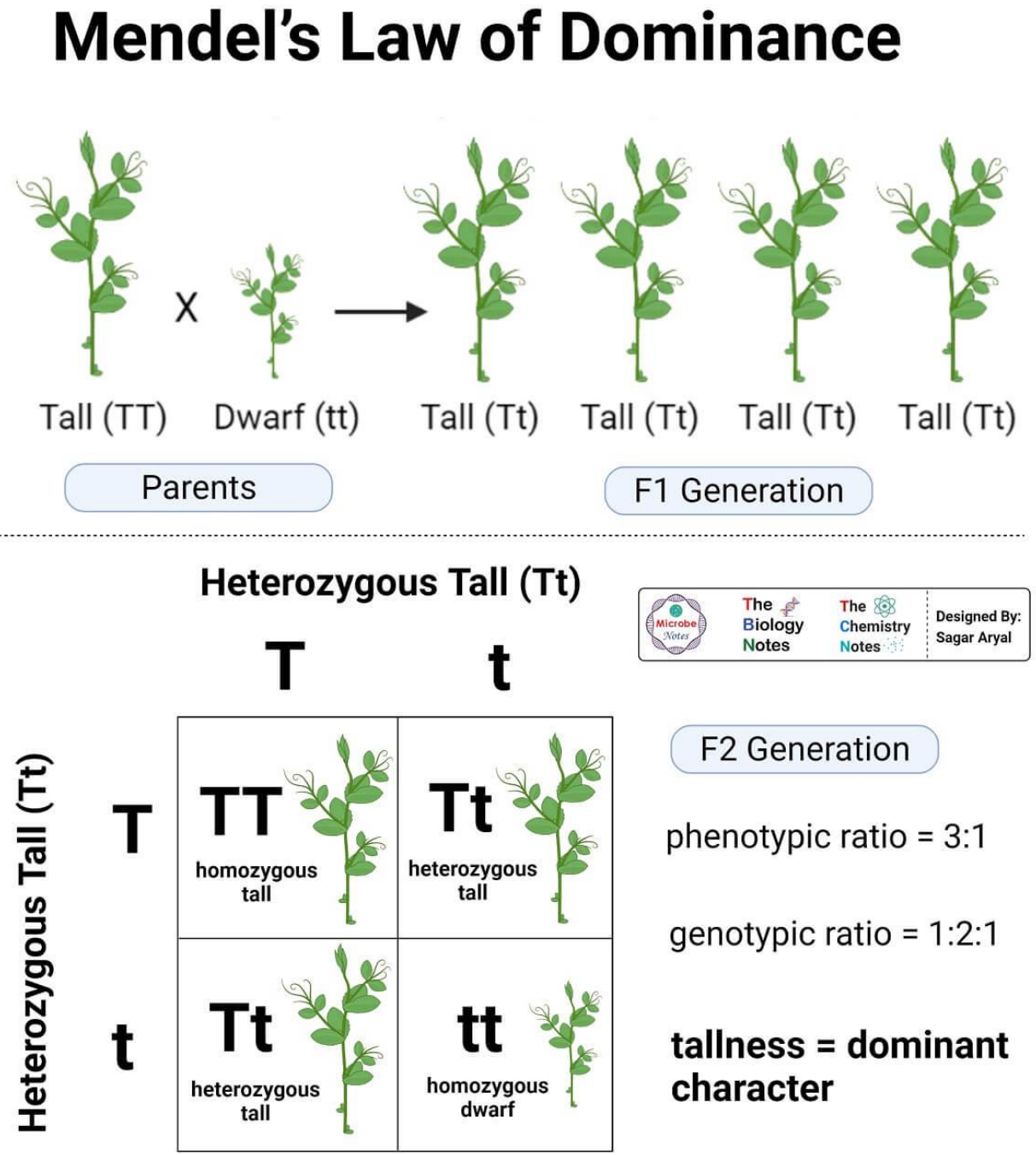
- Remeber MIOSIS Prophase scheme in previous lessons :



THIRD MENDEL LAW (law of dominance)

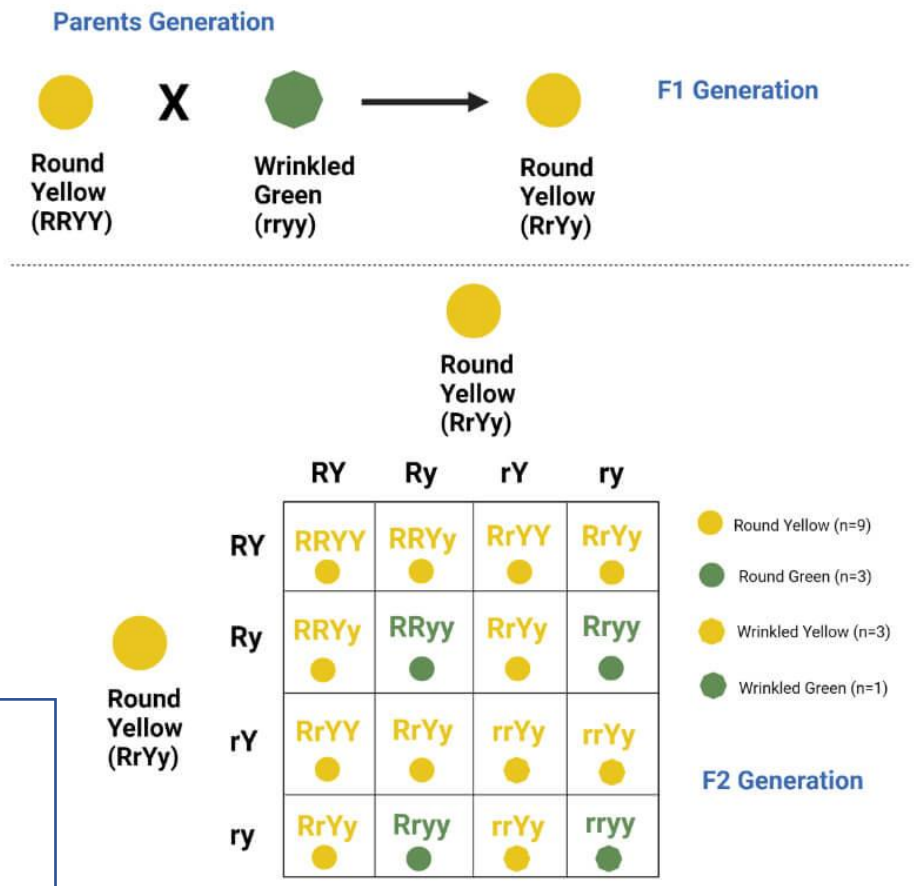
•The pair of genes can be homozygous (TT or tt) or heterozygous (Tt) , and in the case of heterozygous pairs, one of the factors dominates the other.

•The character that dominates (T) is called the dominant character, and the one that remains unexpressed (t) is the recessive character.
•The recessive character, even though latent, is transmitted to the offspring in the same way as the dominant character.
•The recessive character is only expressed when the offspring has two copies of the same allele resulting in a homozygous individual.

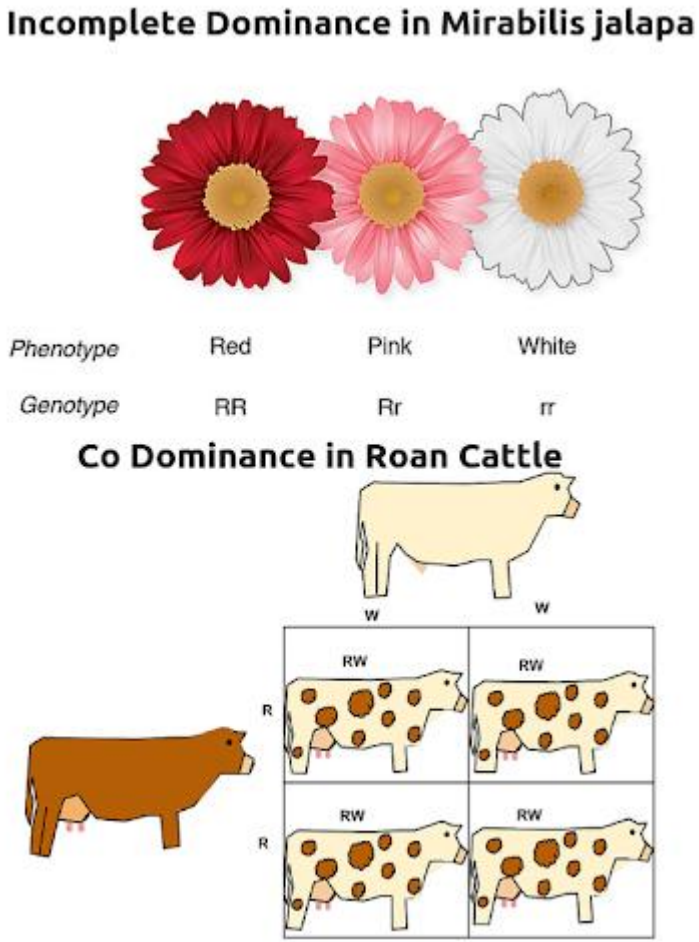


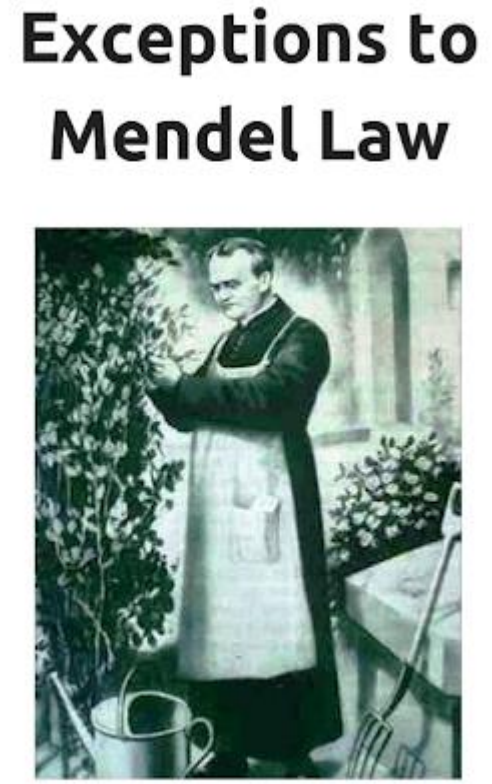
SECOND MENDEL LAW

Mendel's Law of Independent Assortment



Mendel laws have some exception in some organism: example where DOMINANT is not full:





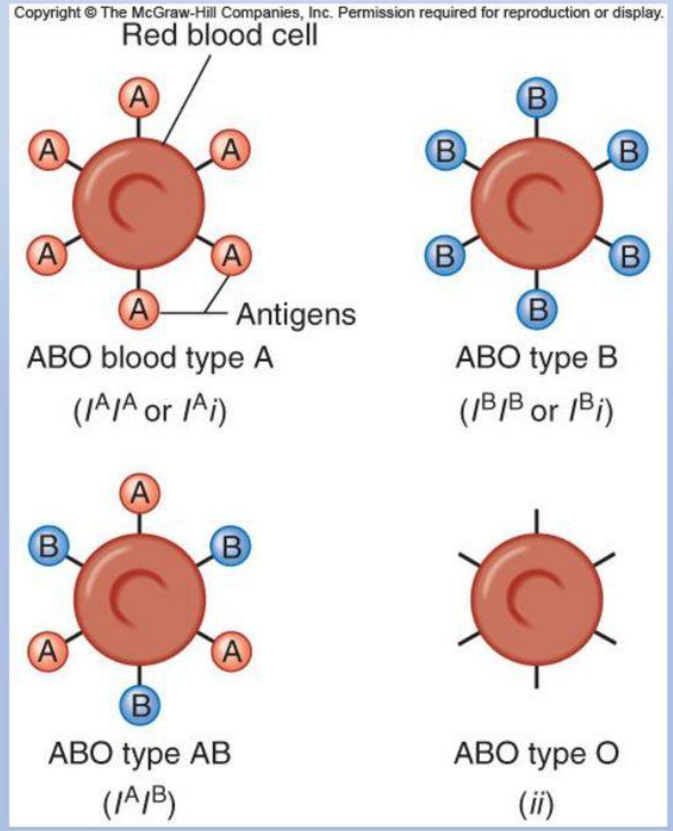
…or some other exception (the case of human surface molecules of erytrocytes)

Mendel's traits showed two distinct forms: either Dominant or recessive

Most genes do not exhibit simple inheritance

Genotypic ratios persist but phenotypic ratios may vary due to "outside-the-gene" influences including

- Multiple alleles
- Other nuclear genes
- Non-nuclear genes
- Gene linkage
- Environment



MEMORIZE some antoher important definiton from basic genetic:

Epistasis

The phenomenon where one gene affects the expression of a second gene

Example: Hairless dogs: genes for color of hair have no effect if not hair is produced. Epistatic interaction seen is albinism in which one gene blocks the genes that produces color

Example: **Bombay phenotype**

Mendel law are used in biotechnology also for precomputing (prediction) of possible dihybrids (combinations of 2 genes:

predictable ratio of phenotypes

	genotype	phenotype	number	phenotypic ratio
• Parent	Y_R_		315	9/16
• Recombinant	yyR_		108	3/16
• Recombinant	Y_rr		101	3/16
• Parent	yyrr		32	1/16

Ratio of yellow (dominant) to green (recessive)=3:1 (12:4)
Ratio of round (dominant) to wrinkled (recessive)=3:1 (12:4)

Final notes to some genetic disorders:

- Not only one gene caused one function (or disfunction)

Genetic Heterogeneity

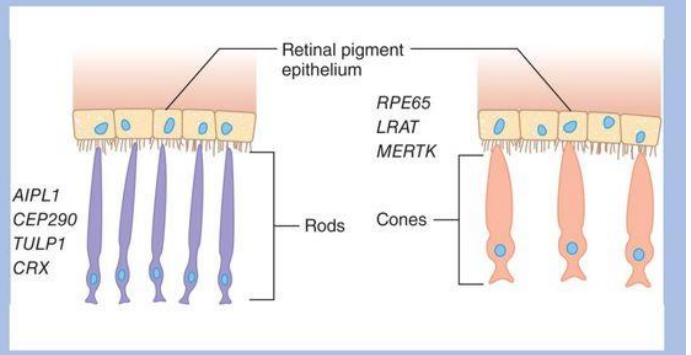
Different genes can produce identical phenotypes

- *Hearing loss* – 132 autosomal recessive forms

- *Osteogenesis imperfecta* – At least two different genes involved. Abnormal collagen causes very brittle bones in children.

- *Alzheimer disease* – At least four different genes involved. Genes may encode enzymes that catalyze the same biochemical pathway, or different proteins that are part of the pathway

- *pathways to blindness* mutations in over 100 genes cause degeneration of the retina resulting in many pathways to blindness



Overview: important PHENOMENON and definiton from more advance genetic theory

Table 5.1 Factors That Alter Single-Gene Phenotypic Ratios

Phenomenon	Effect on Phenotype	Example
Lethal alleles	A phenotypic class does not survive to reproduce.	Spontaneous abortion
Multiple alleles	Many variants or degrees of a phenotype occur.	Cystic fibrosis
Incomplete dominance	A heterozygote's phenotype is intermediate between those of two homozygotes.	Familial hypercholesterolemia
Codominance	A heterozygote's phenotype is distinct from and not intermediate between those of the two homozygotes.	ABO blood types
Epistasis	One gene masks or otherwise affects another's phenotype.	Bombay phenotype
Penetrance	Some individuals with a particular genotype do not have the associated phenotype.	Polydactyly
Expressivity	A genotype is associated with a phenotype of varying intensity.	Polydactyly
Pleiotropy	The phenotype includes many symptoms, with different subsets in different individuals.	Porphyria variegata
Phenocopy	An environmentally caused condition has symptoms and a recurrence pattern similar to those of a known inherited trait.	Infection
Genetic heterogeneity	Different genotypes are associated with the same phenotype.	Leber congenital amaurosis