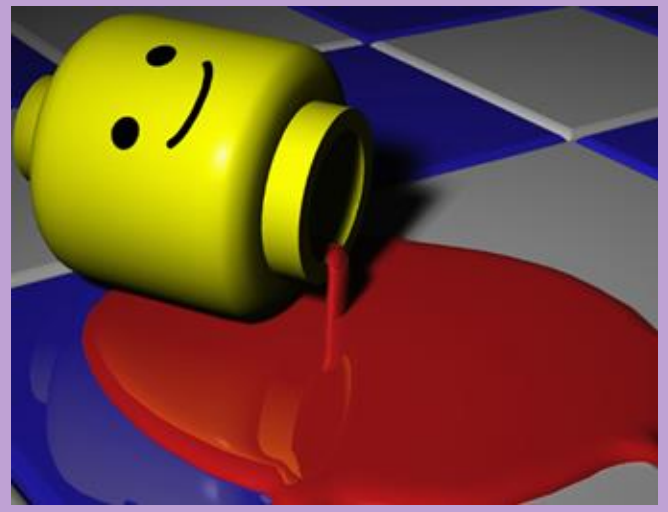
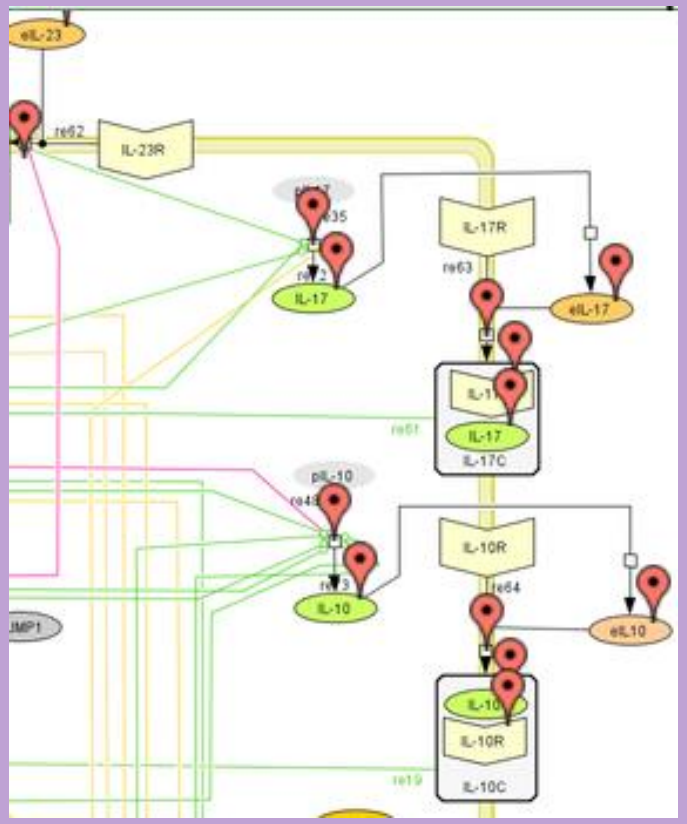
MODERN METHODS

=overview of 3 anti-patogen strategy of cell and medicine





J. Skopalík
14.12.2021

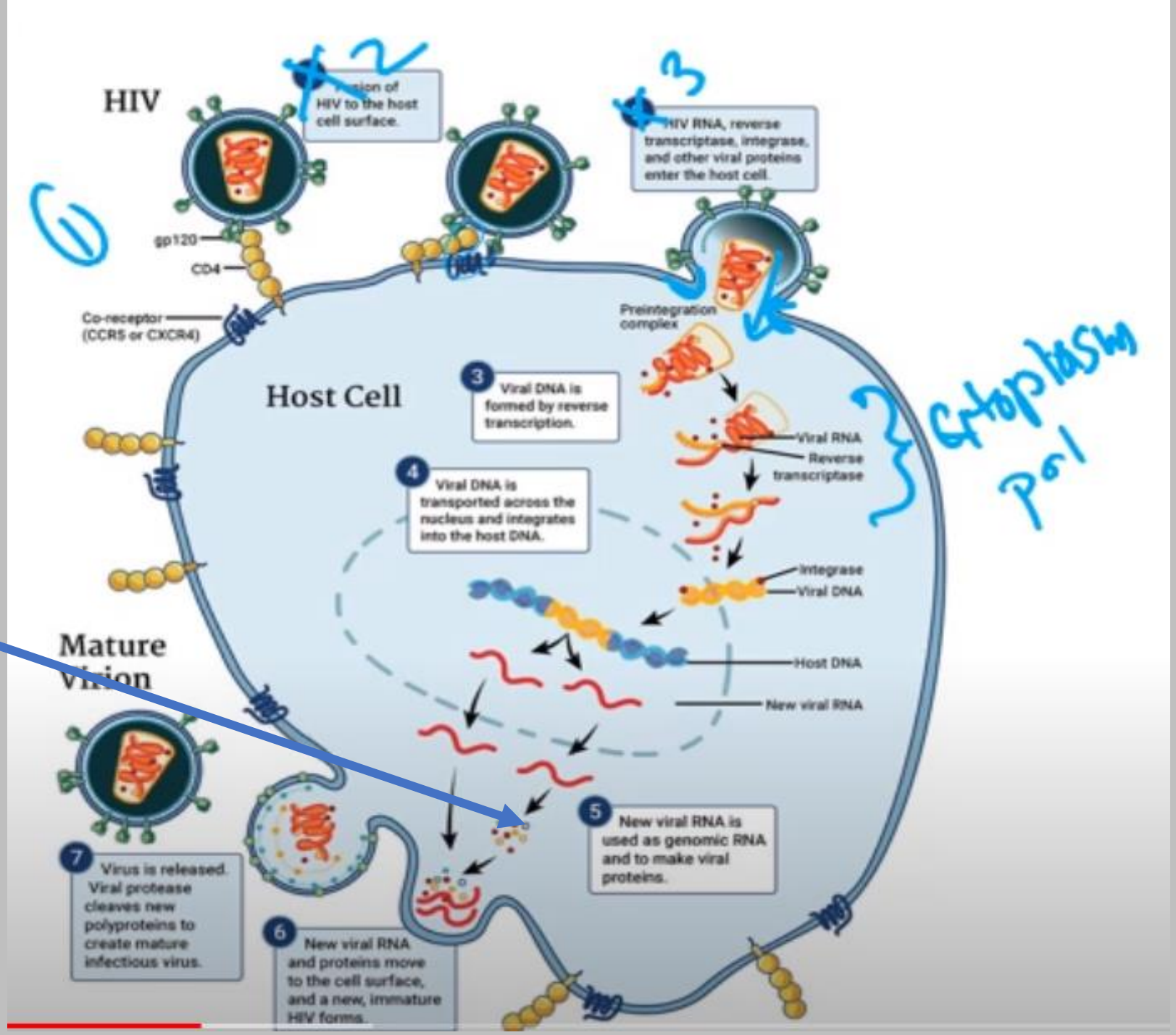
Basic question from practiacal medicine:

- What does patogen (virus) need ????

a) aerosol b) extracelulár matrix c) cytosol ???

a) live cell b) death cell ???

Virus need
live cells.
Virus need cytosol,
where translation
of DNA is activated:



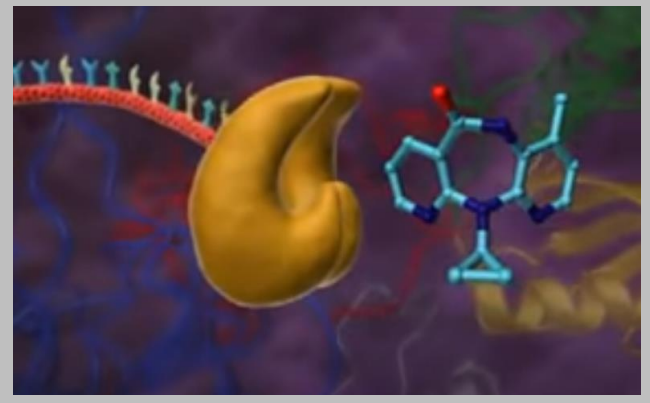
Where are the place of cell defence ?

1) Blocking of VIRUS landing by FUSION INHIBITORS

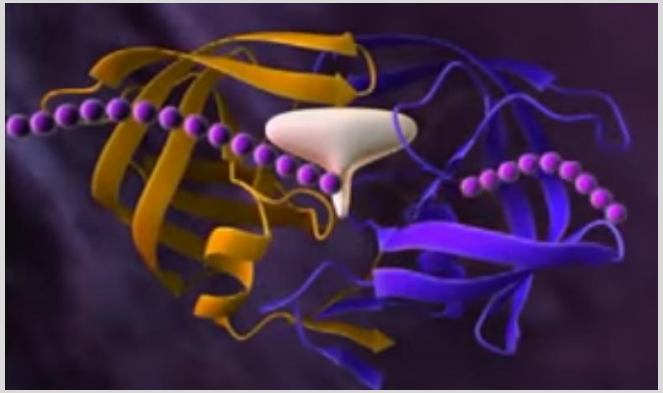
analogy to moon landing

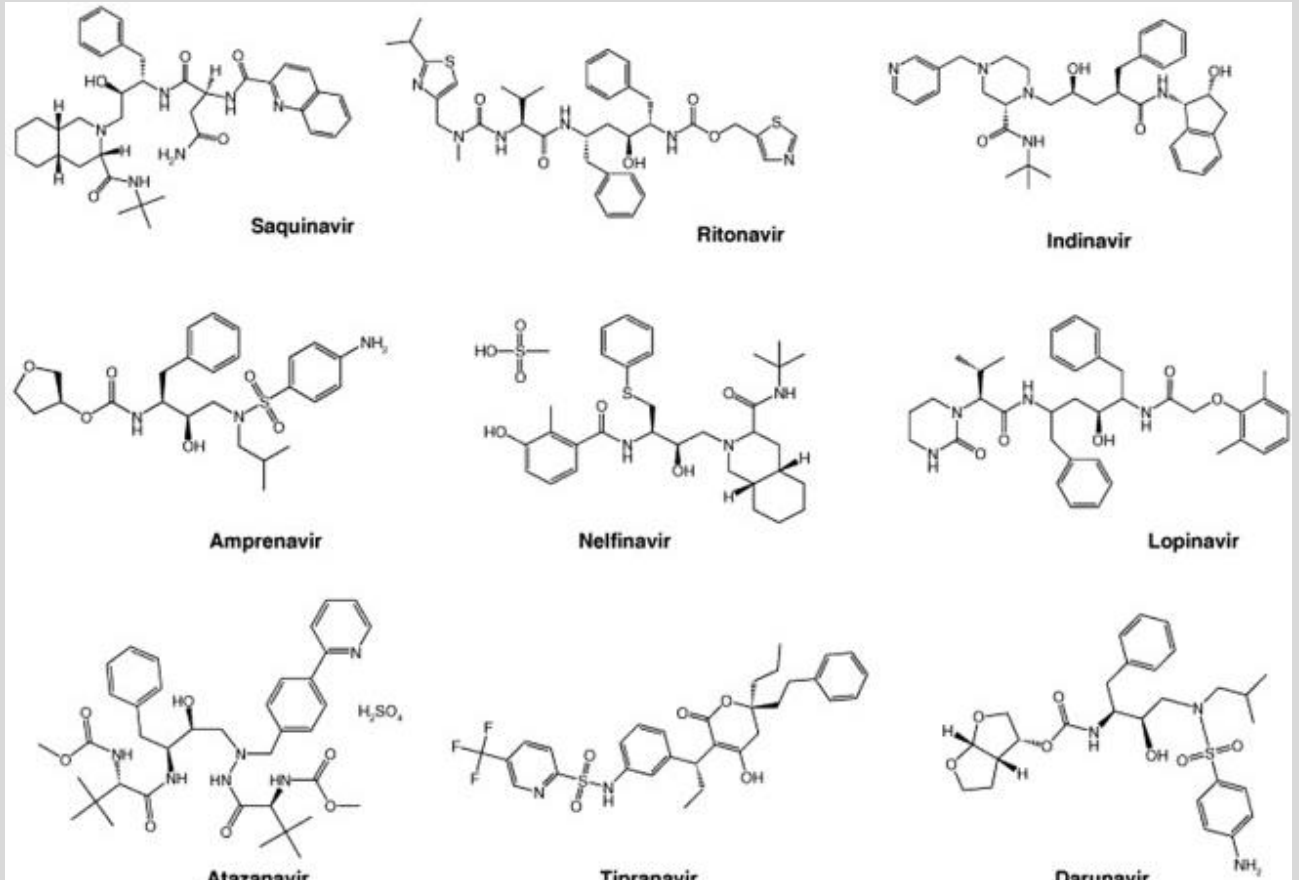
2) Nucloside inhibitors

3) NON-nulceoside inhibitors



4) Protease inhibitors against HIV protease. (Because HIV protease is responsible for processing of the gag and gag-pol polyproteins during virion maturation. The activity of this enzyme is essential for capsid finalisation and escape of virionnfrom cell)





- 5) KILLING OF THE INFECTED CELLS by lymphocytes or antoher immune cells

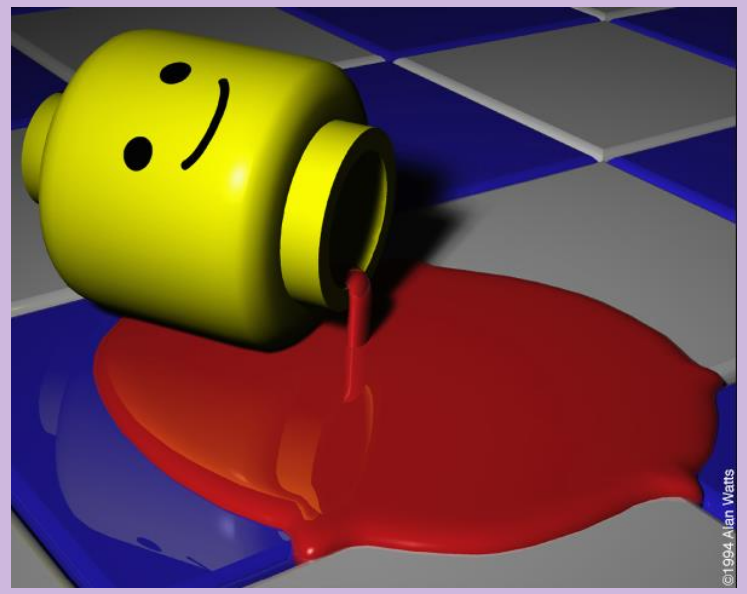
TODAY 3 SHORT EXCURSION TO:

A – KILLING OF THE CELLS (nature immune raction against patogen)

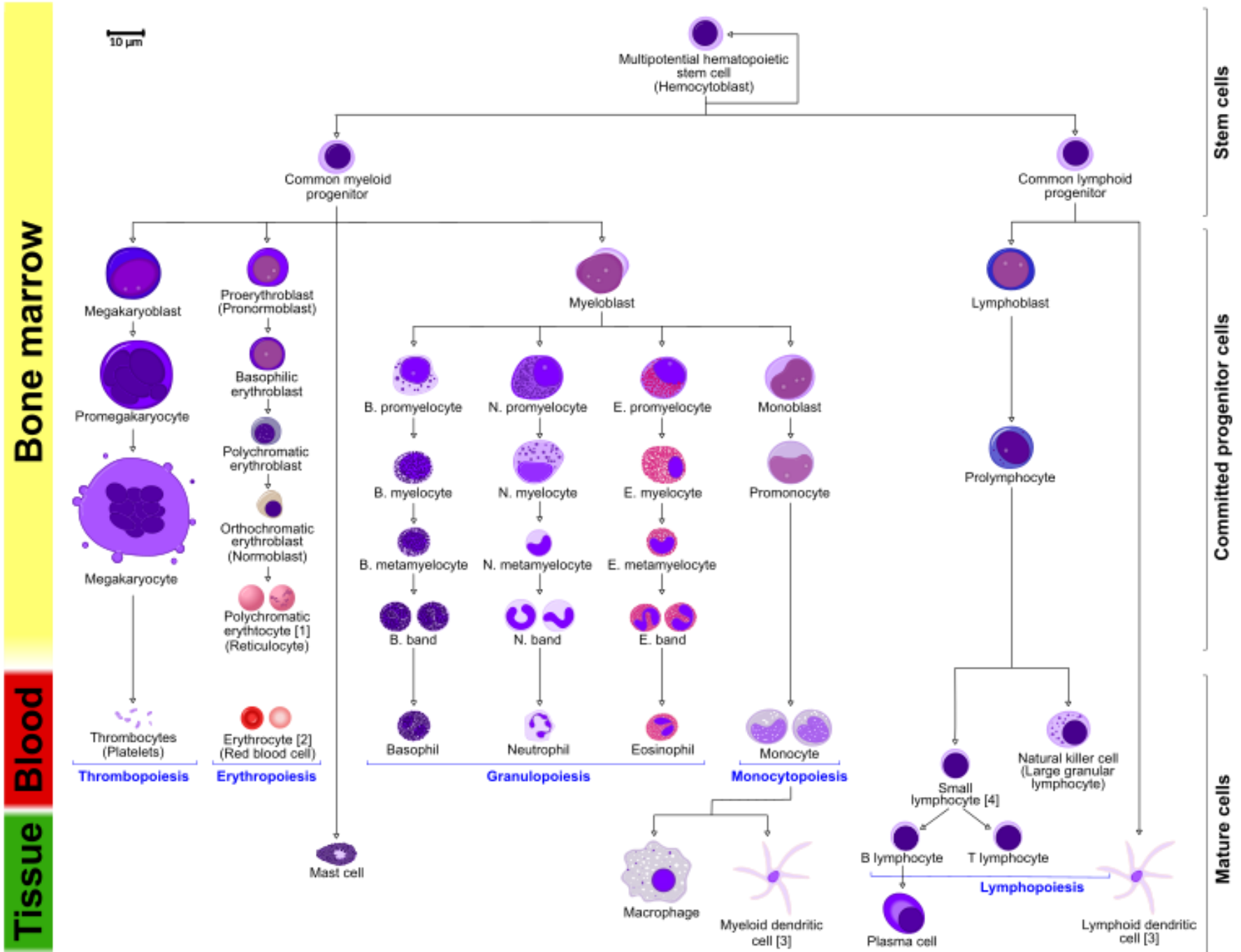
B - FLOW-CYTOMETRY
(machine for T cell and another cell evaluation in patients)

C – IN SILICO design of ENZYME INHIBITORS (drug which stop not only the HIV virus) IN SILICO versu IN VIVO realitě)

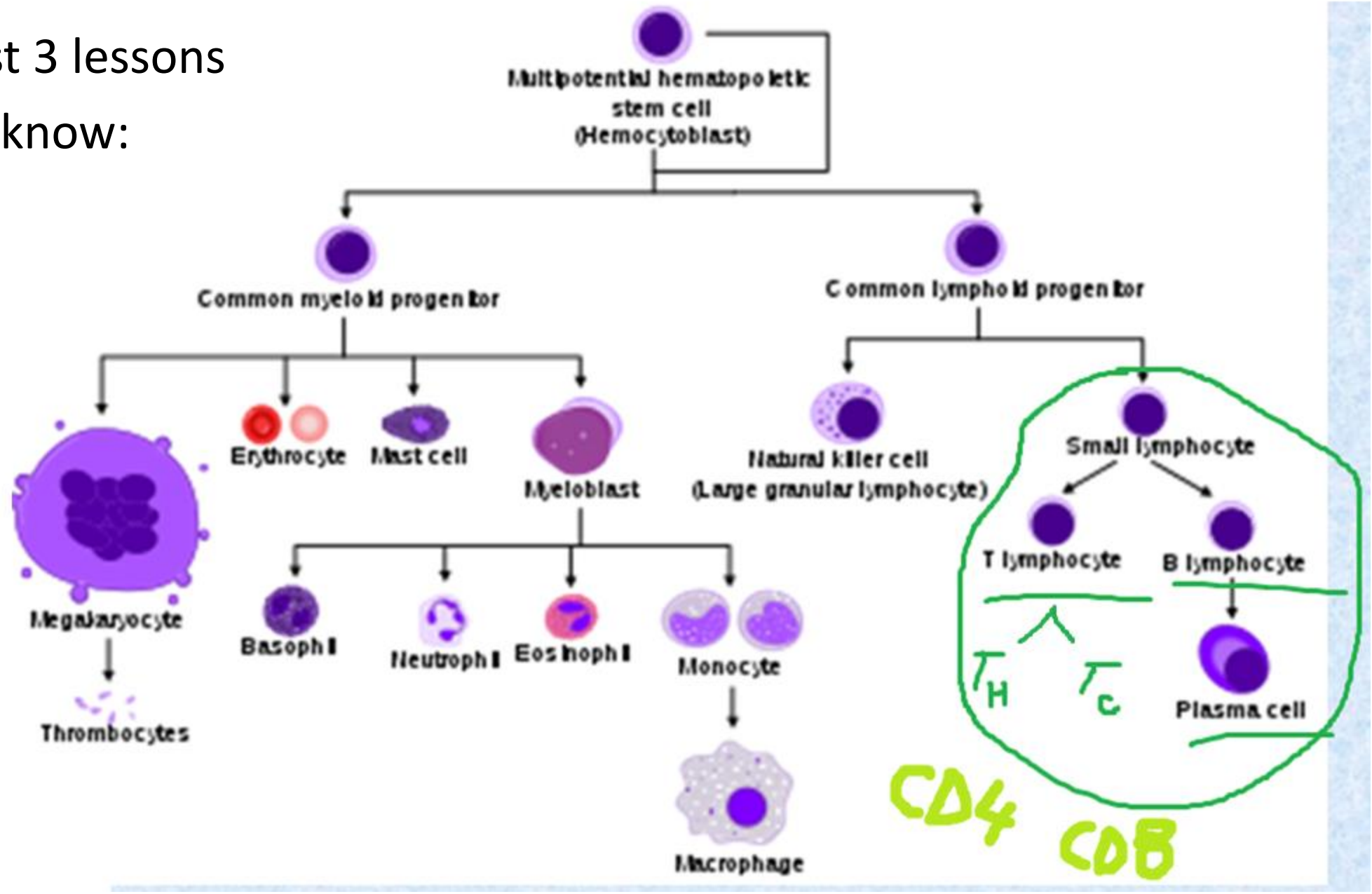
A – KILLING OF THE INFECTED CELLS



From last 3 lessons you can know:



From last 3 lessons you can know:

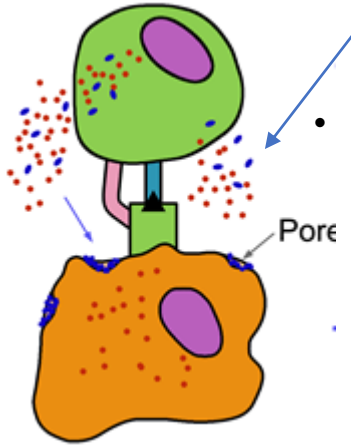


T cell

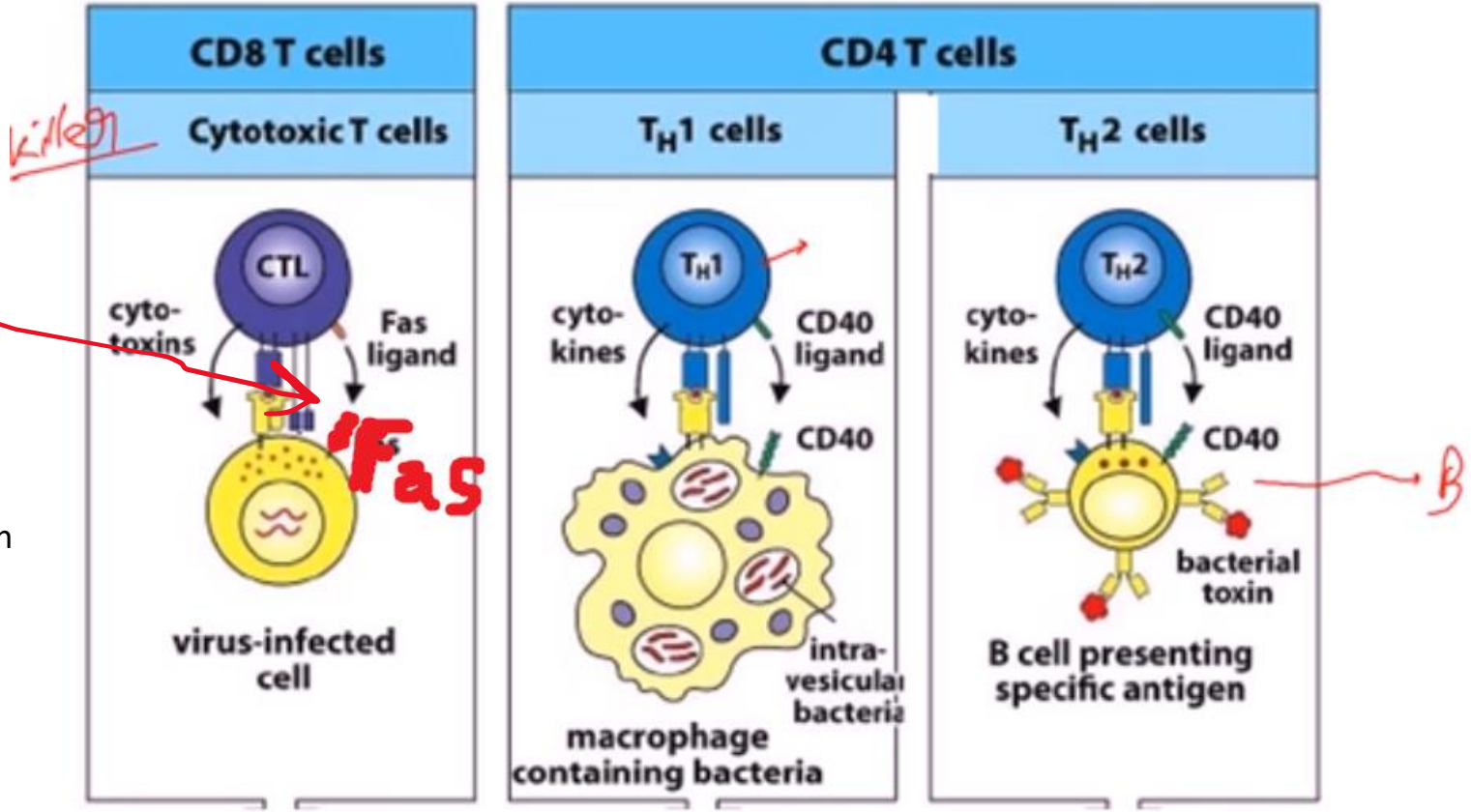
Cytotoxic T-CELL are the Most effective „killing cells“. They can recognise the infected cell, and they can start cell death (by FAS ligand, or by perforins)

(dont forgot that

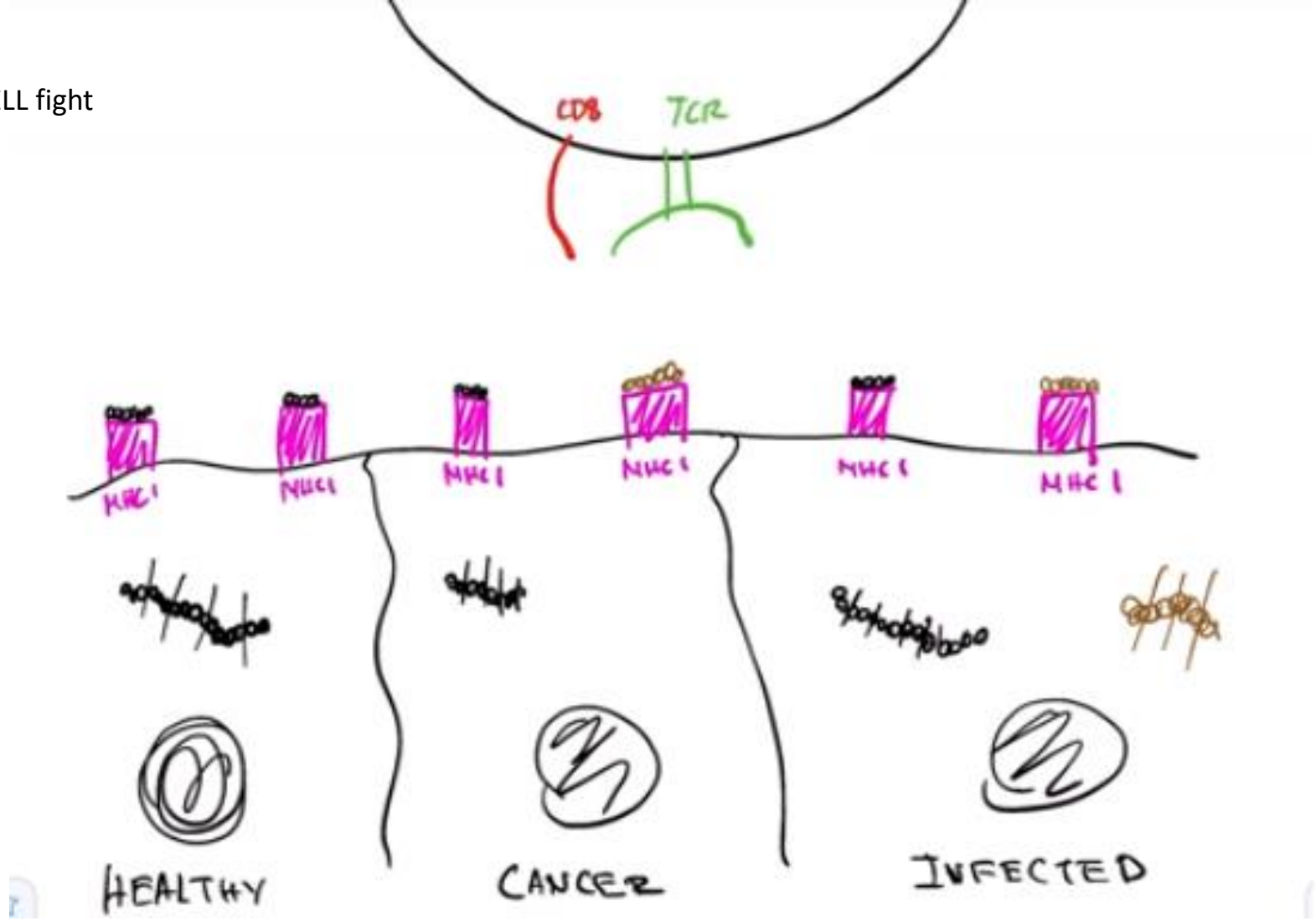
also some another T cells exist: Th1 and Th2)



- Perforins casused perforation of cell mebranes and cell death ane elimination of virus replication

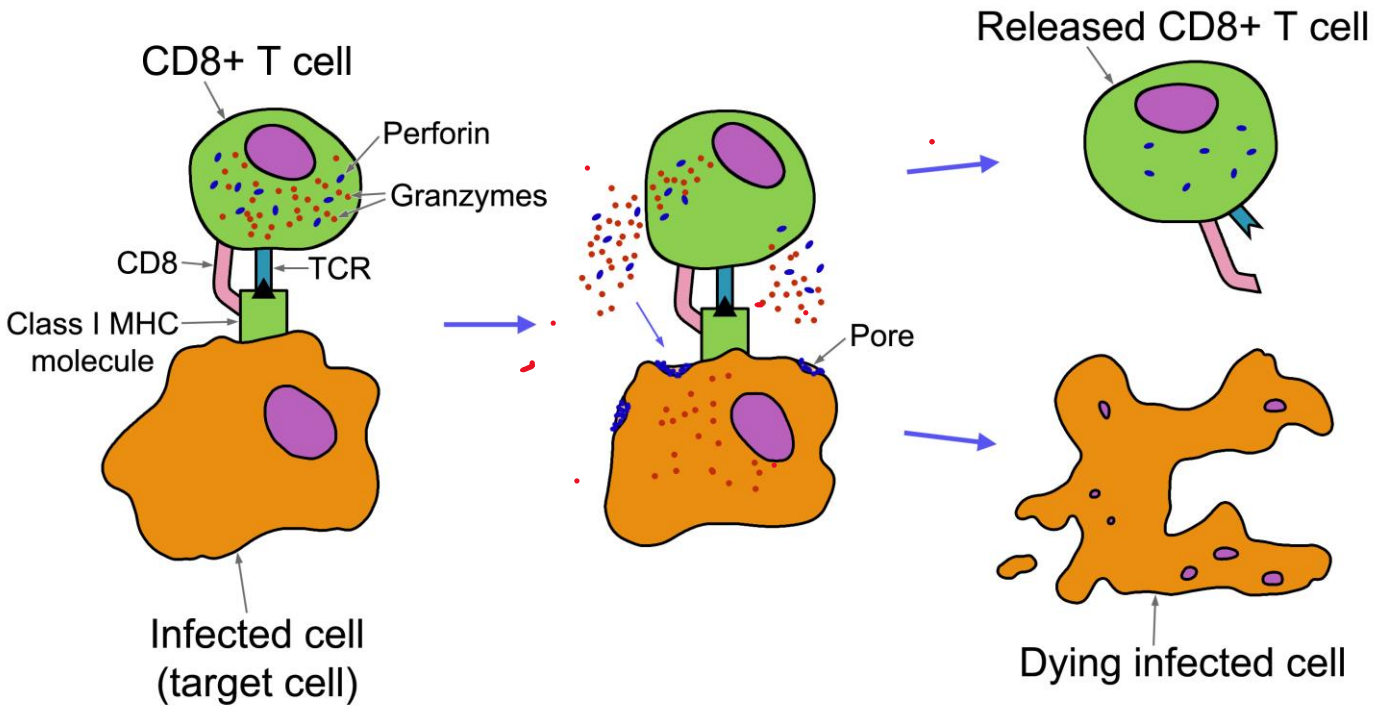


Very good overview of kinetic of CYTOTOXIC TCELL fight against CANCER or INFECTED CELLS:

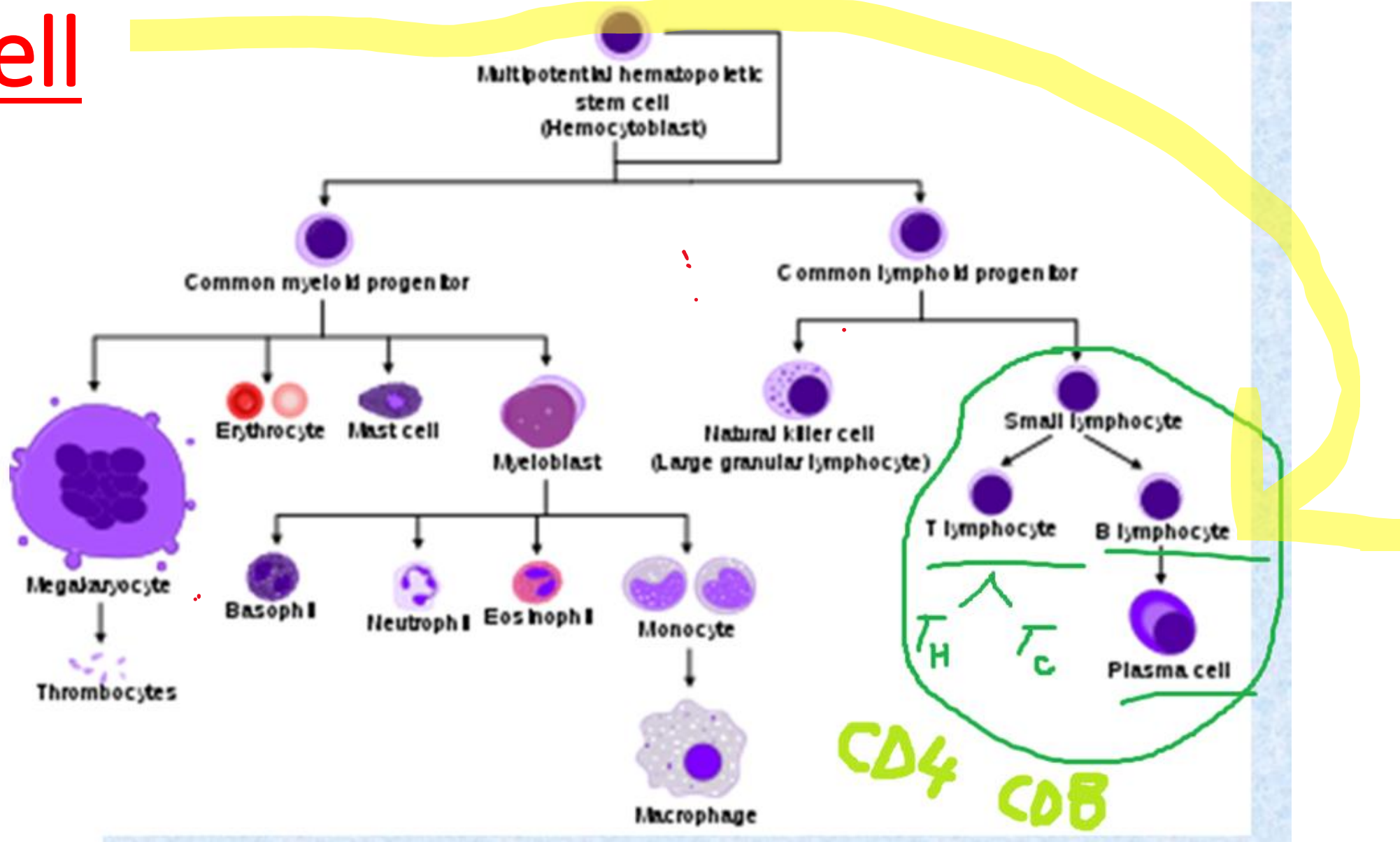


See the online movie:
Cytotoxic T Cells and T Helper Cells - Bing video

Scheme of the T-lmyhpocyte perforin action from textbook (Alberts 2003)



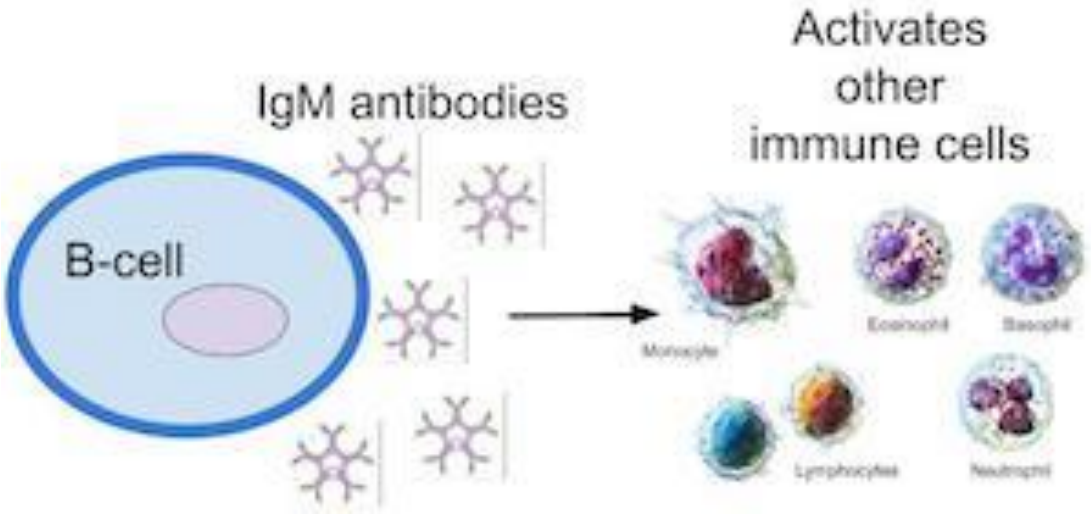
B cell


Multipotential hematopoietic stem cell (Hemocytoblast)
Common myeloid progenitor
Common lymphoid progenitor
Erythrocyte
Mast cell
Myeloblast
Natural killer cell (Large granular lymphocyte)
Small lymphocyte
T lymphocyte
B lymphocyte
Megakaryocyte
Basophil
Neutrophil
Eosinophil
Monocyte
Plasma cell
Thrombocytes
Macrophage
CD4
CD8

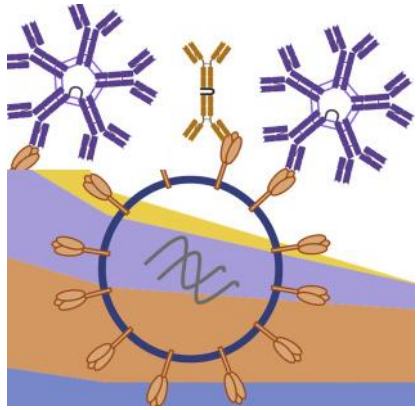
- B cells produce IgM antibodies.

IgM antibodies are pentamers, which have two main effect:

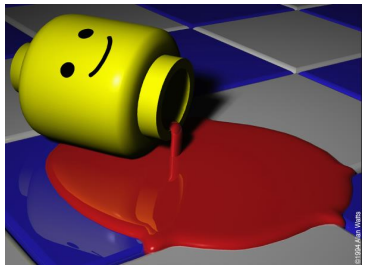
1) activation of another immune cells,



2) direct connectionm and neutralisation of viruses and bacteria



Result:



Result:

The IgM antibody is the largest antibody and the first to appear to fight off a new infection. The IgM is basically the first line of immune defense against fighting pathogens. In inflammatory diseases, the IgM can have both pathogenic and protective roles depending on the type of infection and tissue affected. IgM is present in jawed vertebrates (gnathostomes) that existed already during the Devon period over 400 million years ago. An IgM antibody test is very important because it generally comes up earlier on an infection, and it is detectable 4 to 7 days after an infection starts.

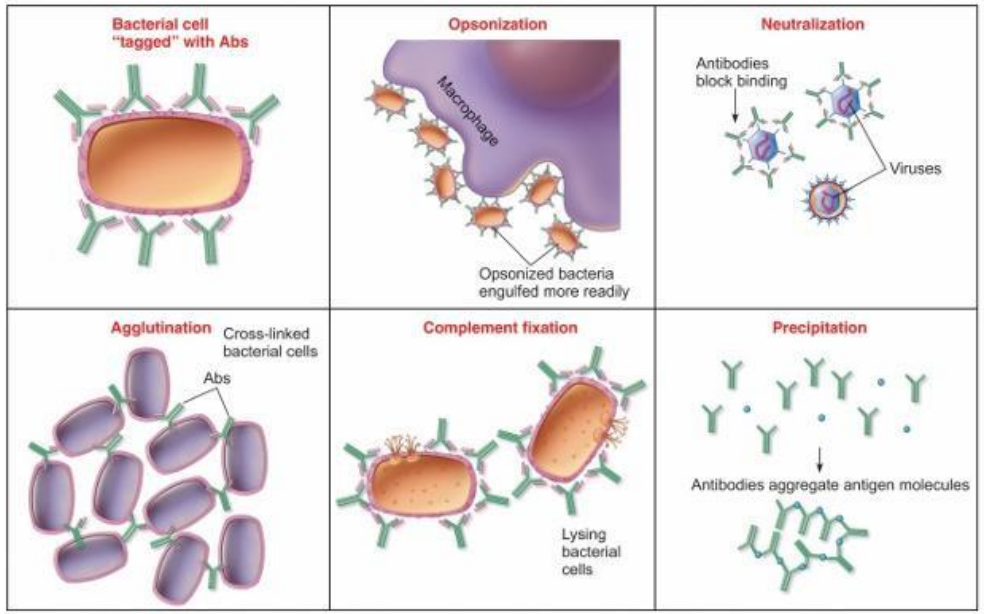
Read more: Difference Between Antibody Test IgG and IgM | Difference Between http://www.differencebetween.net/science/difference-between-antibody-test-igg-and-igm/#ixzz7FEV3j9wQ

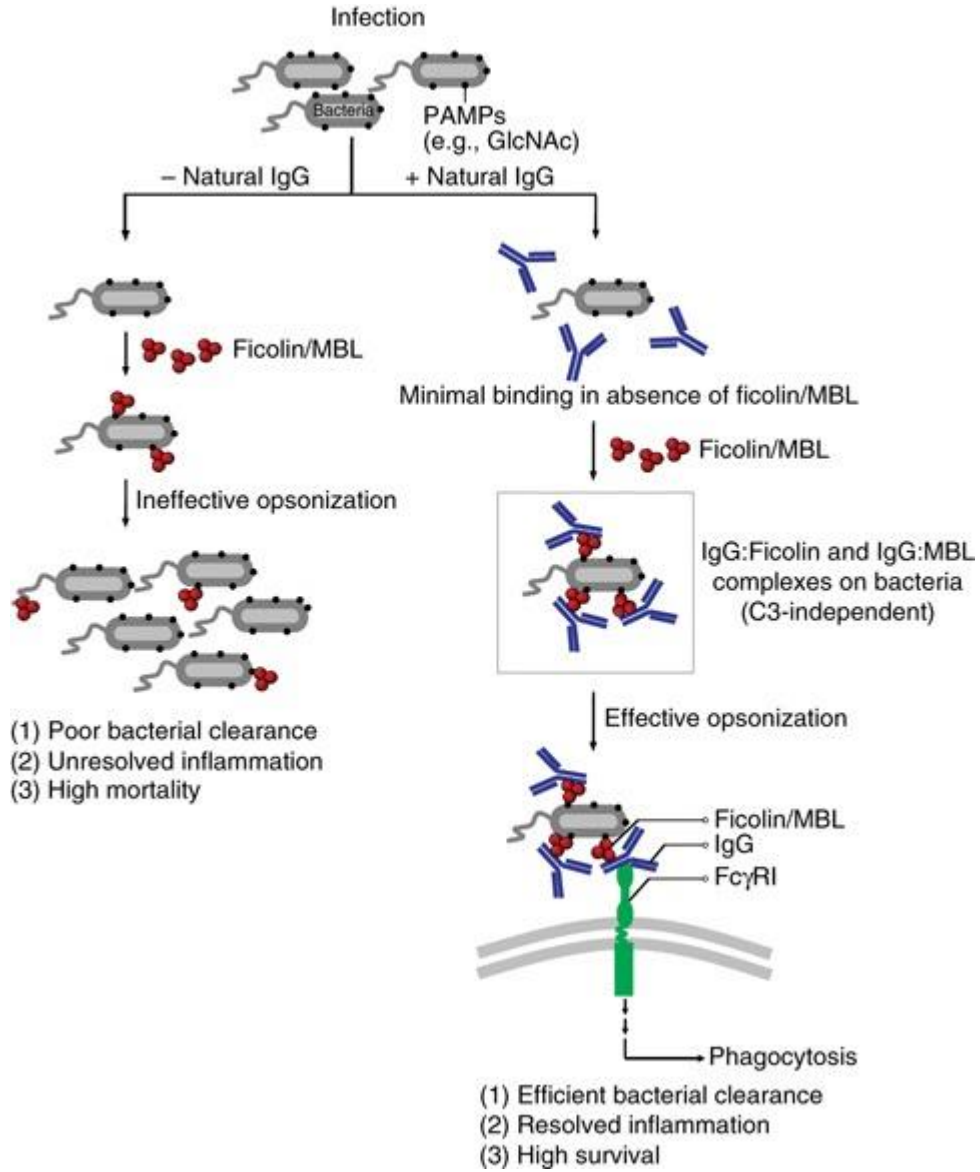
IgG antibodies are formed later than IgM antibodies and the test for IgG antibodies help determine the immunity status following a virus infection or active immunization. It can also help to diagnose persistent infection. So, IgG starts spiking as IgM starts coming down.

Read more: Difference Between Antibody Test IgG and IgM | Difference Between http://www.differencebetween.net/science/difference-between-antibody-test-igg-and-igm/#ixzz7FEUm5IGV

Also IgG antibodies, have similar function like IgM:

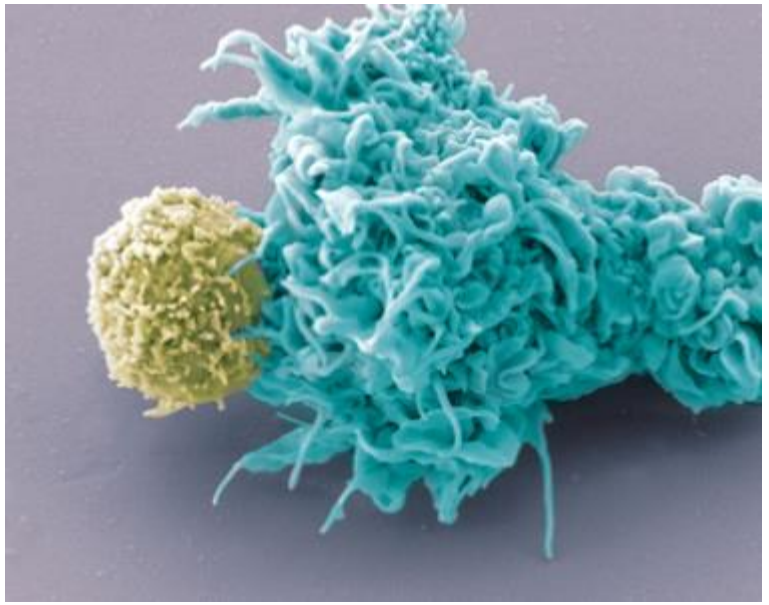
- Produced antibodies bind antigens and stop their destructive behavior in one of several ways





Some microscopic photo of immune cells contact to patigen or cell-cell contact:

\macrophage bacterium c4d



T lymph. / dendritic cell

B -FLOW CYTOMETRY

- Flow Cytometry is a technique used to detect **and measure physical and chemical characteristics of a population of cells or particles**.
- In this process, a sample containing cells or particles is suspended in a fluid and injected into the flow cytometer instrument. Detectors detect if each one cell have CD8 or another surface molecule. And computer compute how many CD8 positive cells are in the sample and for example how many CD4 and CD90 and CD 73 positive ….

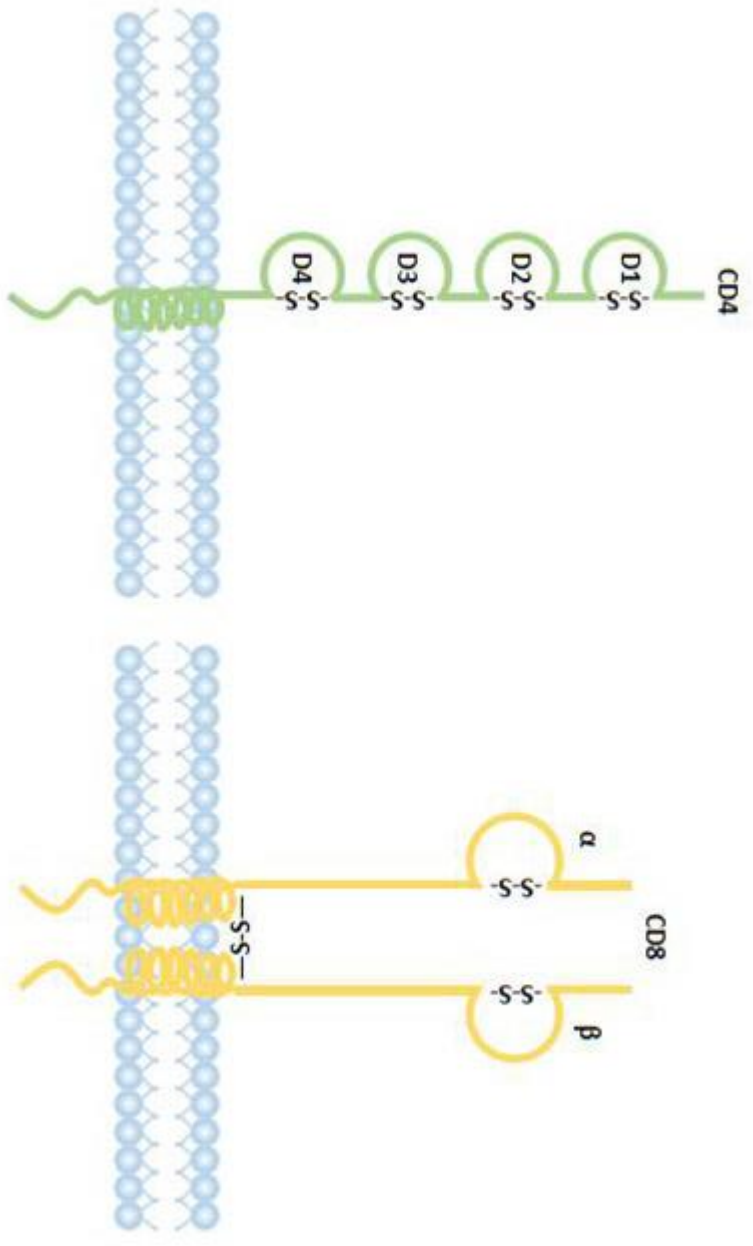
CD4

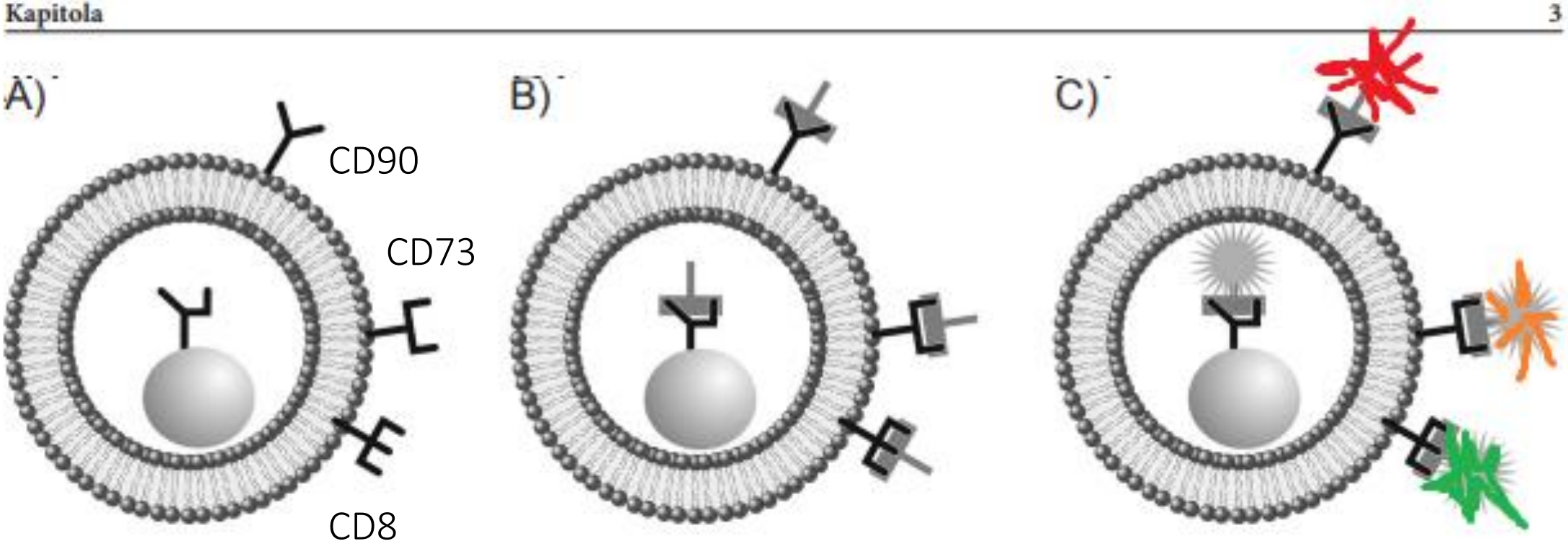
CD4 is a T helper cell marker, which is a single chain transmembrane protein. The extracellular structure belongs to IgSF, and there are four IgSF domains. The first and second domains can bind to MHC class II molecules. CD4 acts as a co-receptor for the TCR-CD3 complex recognition antigen and participates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

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.





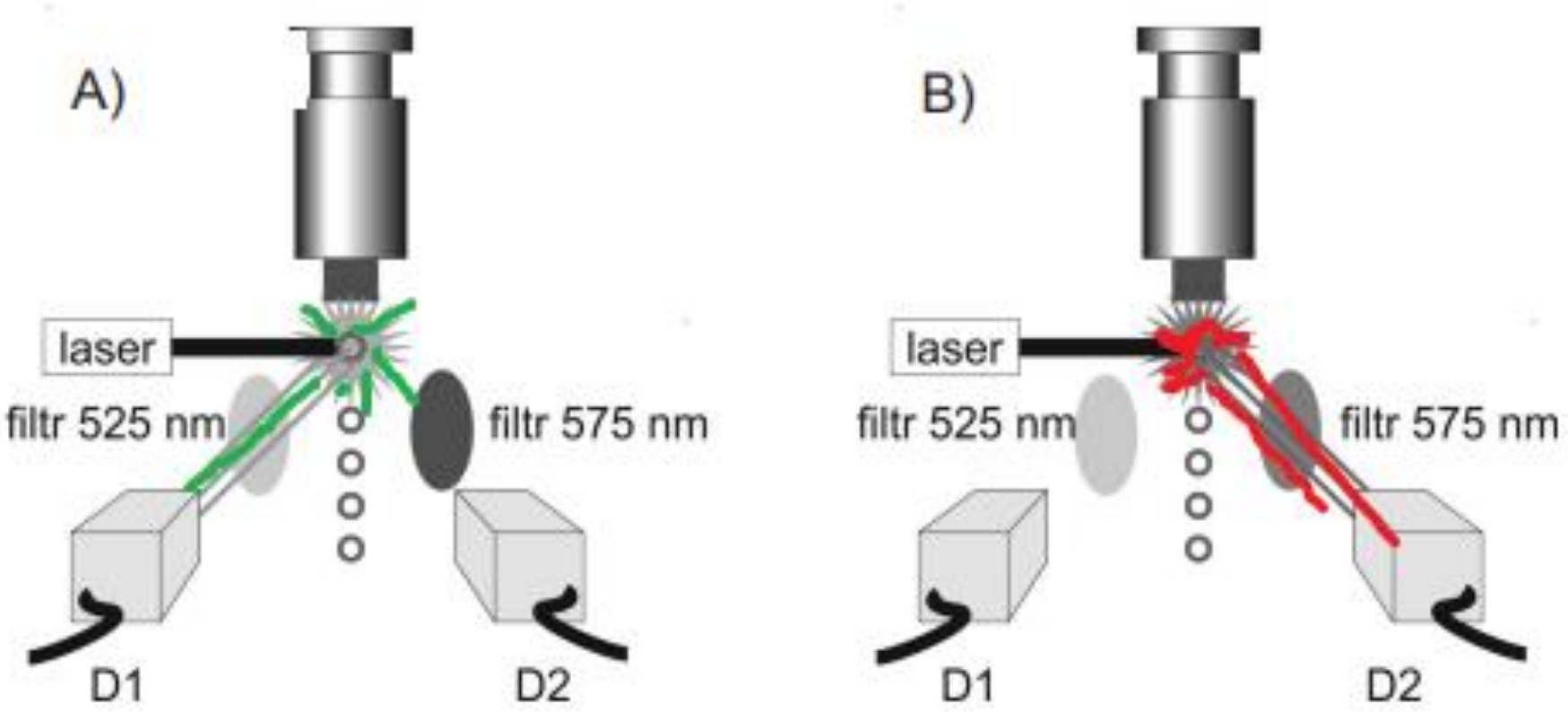
Set of surface molecules.

Specific antibody

Antibody + fluorescence molecule

Detectors detect if each one cell have CD8 molecules (GREEN FLUORESCENT)

Or CD 90 for example (in this case: red fluroescence).

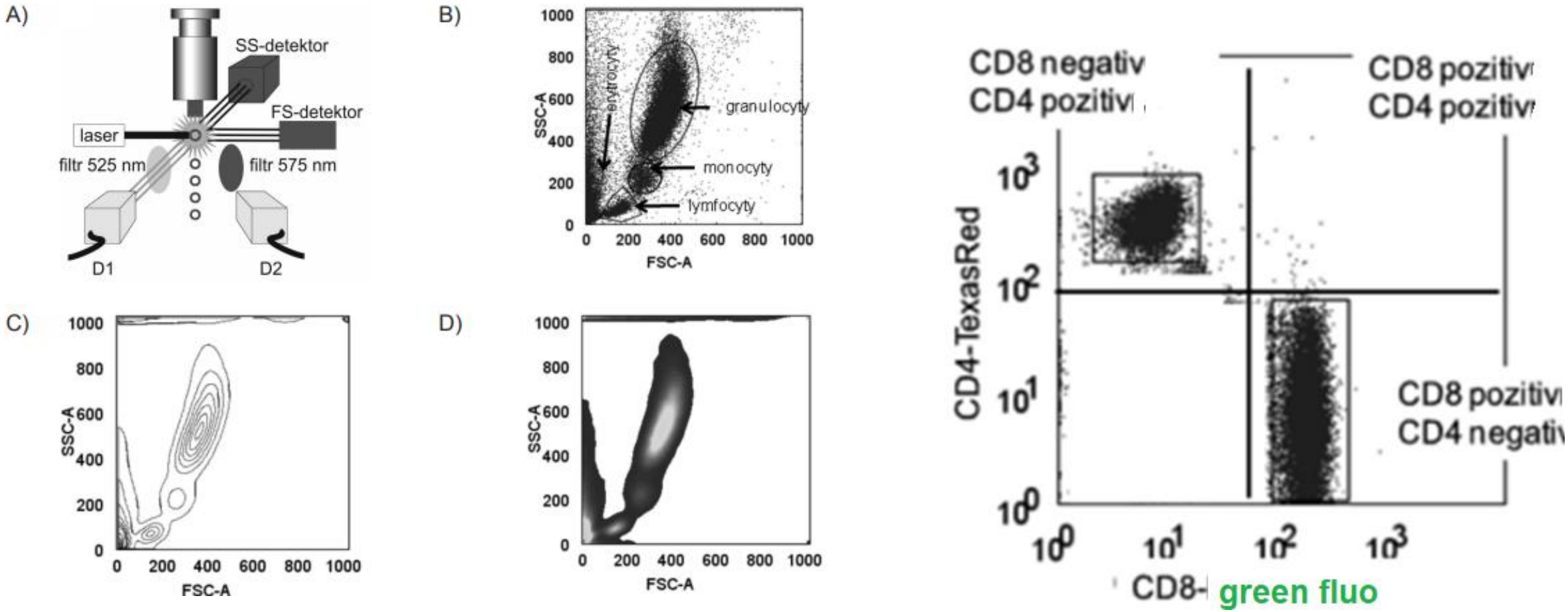


Obr. 3.14.2. Základní princip fluorescenčního modu průtokového cytometru – suspenze buněk je protlačována velmi ma-

Modern multiparametric cytometry (result presented on typical PC software protocol)

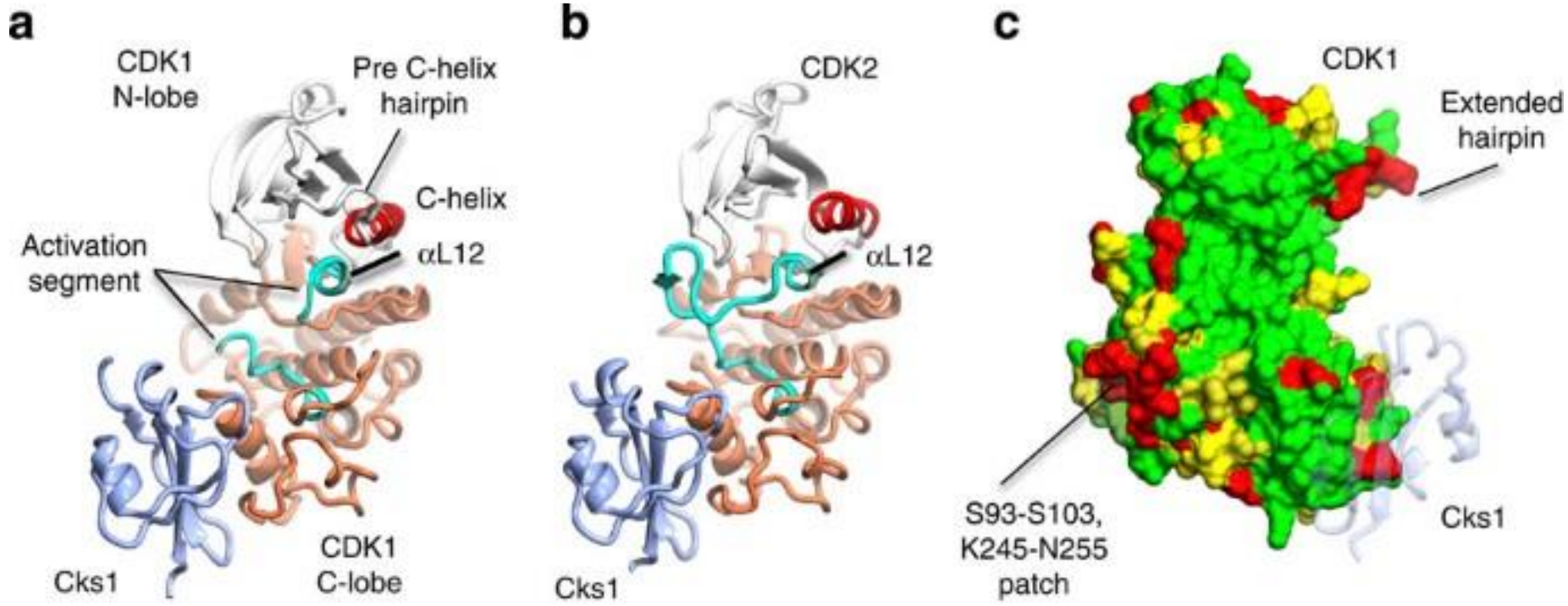
size of cell statistic:

CD8 positivity statistic:

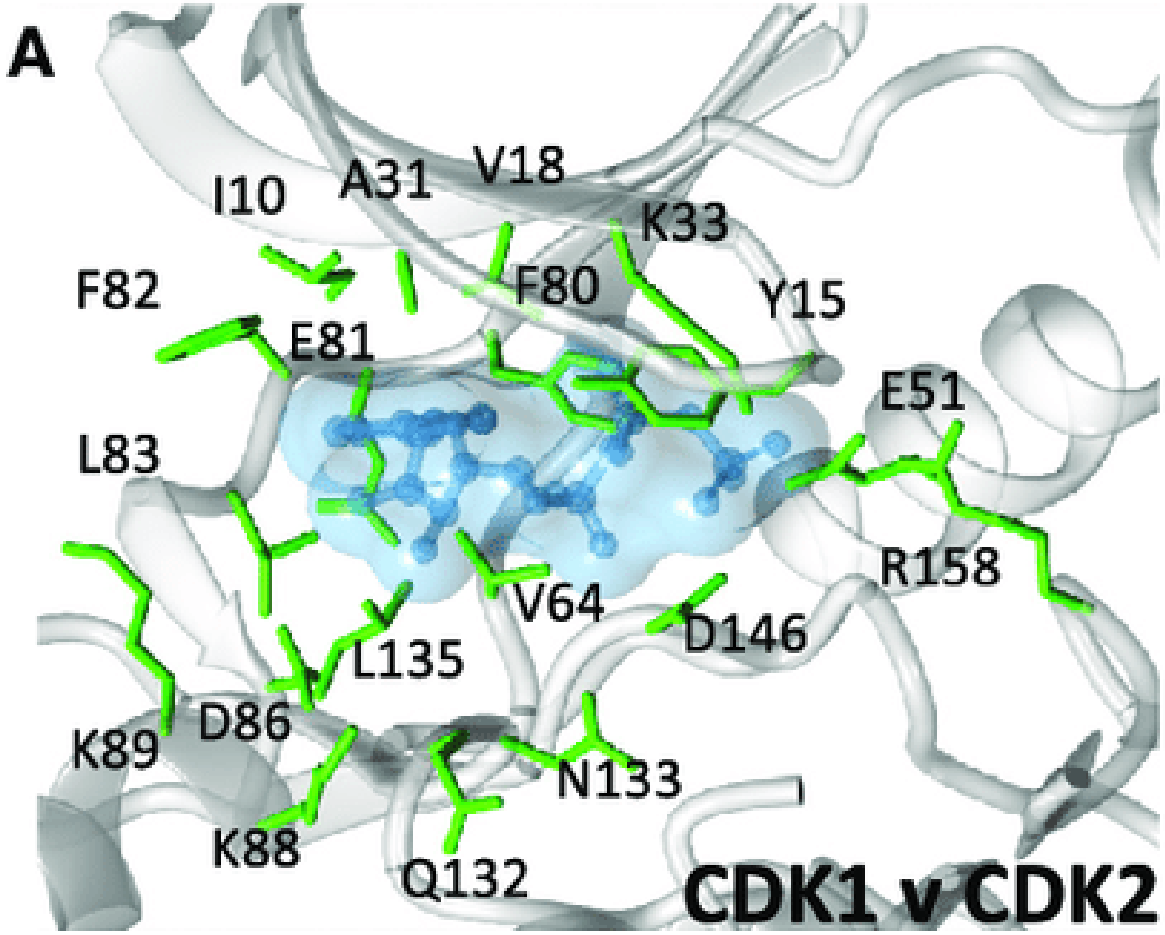


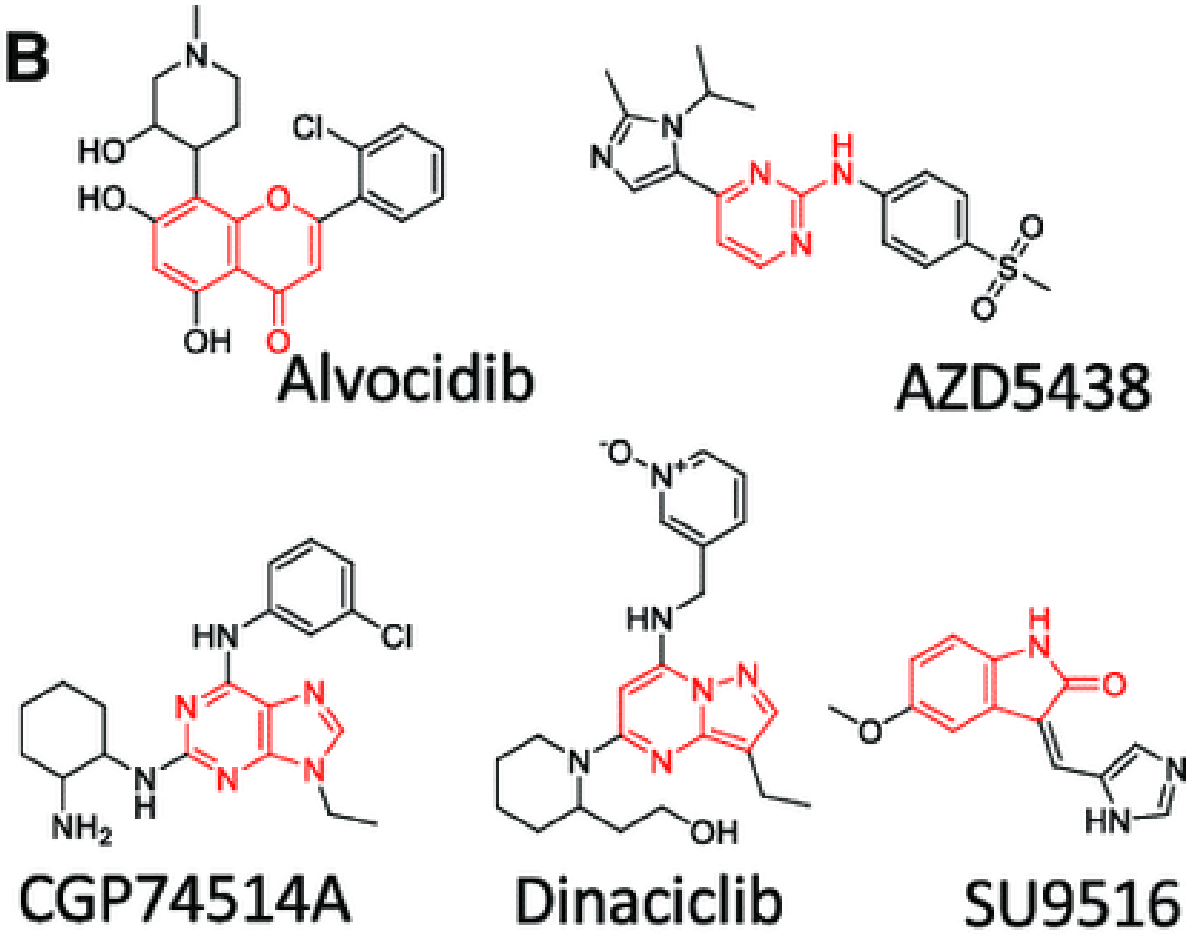
C –
IN SILICO inhibitors desing

At first step: atom 3D map of protease (or another enzyme) had to be build:



At second step: computer asisted docking of molecules into the active site of enzyme („looking for efective pieces of puzzle, which is shape compatible with suroundig structure“)

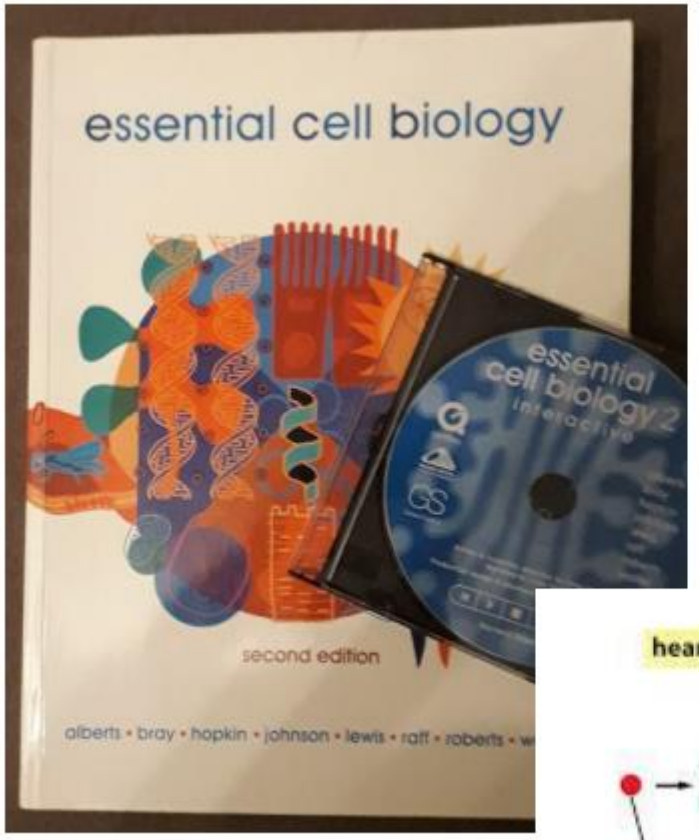




- Effective inhibitors can „click“ to enzyme and „stop“ the enzyme activity (for example they stop the protease which produce viral capsid protein = HIV virus is not able to replicate in the cell and HIV reproduction is stop)

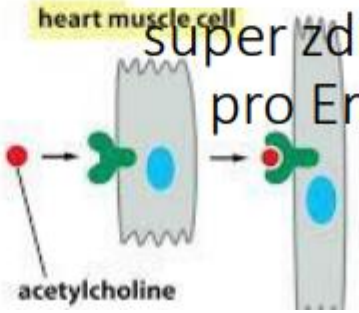
- (after founding of good molecules IN SILICO, the IN VITRO and IN VIVO test had to be started before use in practical human medicine)

The last note to BIOLOGY lessons:



Alberts: Essential cell biology

Big book, dont read all captrures.
Very good for another advace courses of pharmacology.
Very good ilustrative scheme.



super zdroj angličtiny pro Erasmus atd