IMMUNOLÓGIAI ÉS BIOTECHNOLÓGIAI INTÉZET

11th practice: Immune response against pathogens

Basic Immunology

University of Pécs, Clinical Center
Department of Immunology and Biotechnology
Pécs

Main tasks of the immune system

Preserving the integrity of an organism

Defense against **external pathogens** (e.g. viruses, bacteria, parasites)

Elimination of **pathologically altered cells** (e.g. virally infected cells, cancer cells)

Altered foreign structures must be **recognized** and **distinguished** from the organism's own healthy cells.

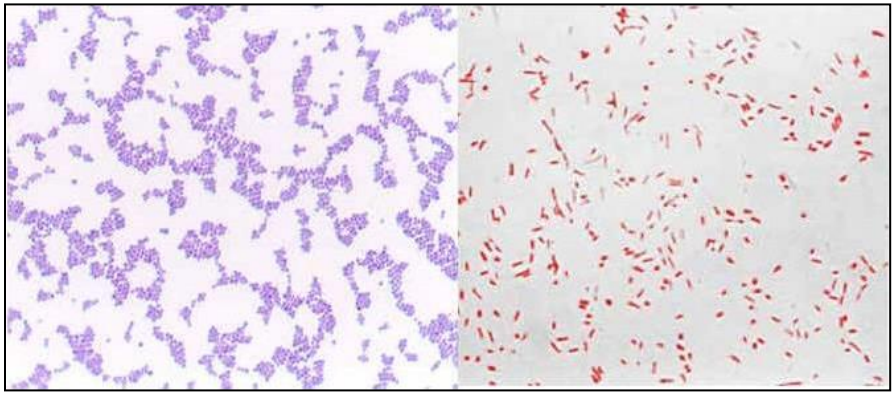
IMMUNE RESPONSE (either an aggressive response or immunological tolerance)

ATTENTION! The **names of some pathogens** are shown on the slides as examples. You **don't have to learn them** for your immunology exam, focus on the mechanisms presented!

What threatens us? I.

1. Bacteria

Gram-positive **Gram-negative**



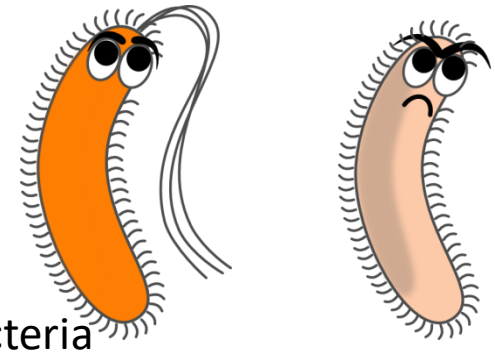
E.g.:

Staphylococcus aureus,
Streptococcus pneumoniae

Escherichia coli,
Salmonella enterica

The **Gram staining** is used to differentiate bacteria based on the **chemical properties of their cell walls**.

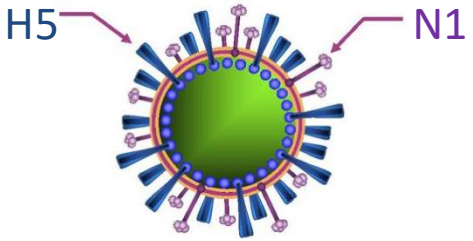
Not all bacteria cause diseases in healthy individuals with a well-functioning immune system, but almost all bacteria can be pathogenic in immunocompromised patients.



Human Microbiome Project: Approx. 10.000 species of bacteria reside in the human body.[1.] (roughly **10^{14} bacteria**, whereas the human body consists of **$3{,}7 \times 10^{13}$** cells[2.])

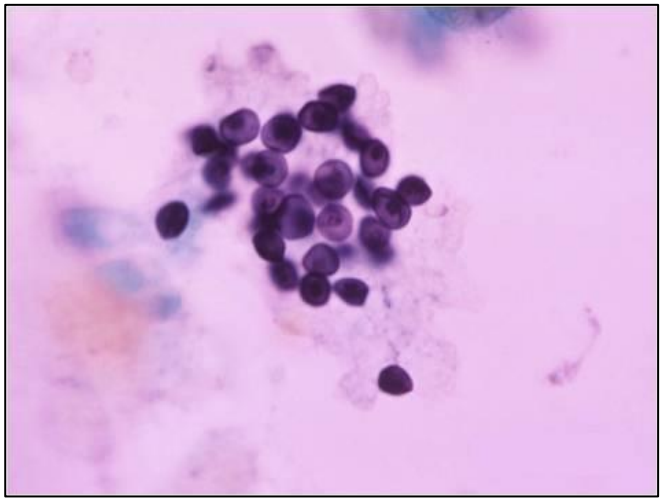
What threatens us? II.

2. **Viruses** (components: single or double stranded nucleic acid chain, outer protein coat which is called capsid)
   - **DNA viruses** (e.g. Herpes viruses, HPV)
   - **RNA viruses** (e.g. Influenza viruses)



H5N1 Influenza virus

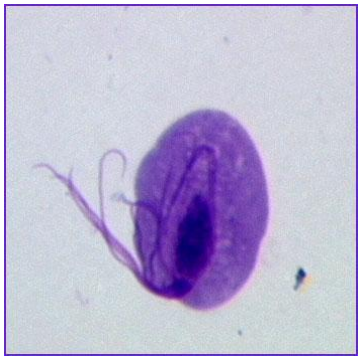
3. **Fungi**
   - Roughly 1,5 million species of fungi live on Earth with approx. 300 being pathogenic to humans.
   - Severe fungal infections mostly occur in **immunodeficient patients**.[3.]



Pneumocystis jirovecii cells in the sputum of a patient with AIDS.[4.]

What threatens us? III.

4. **Protozoa** (**unicellular eukaryotic** parasites), e.g.:
   - *Plasmodium* species → **Malaria**[5.]
   - *Trichomonas* → Vaginitis, urethritis[6.]
   - *Toxoplasma gondii* → Toxoplasmosis[7.]



The flagellated *Trichomonas vaginalis,* causative agent of Trichomoniasis which is the most common non-viral STD with 248 million cases each year worldwide.[9.]

5. **Multicellular parasites**

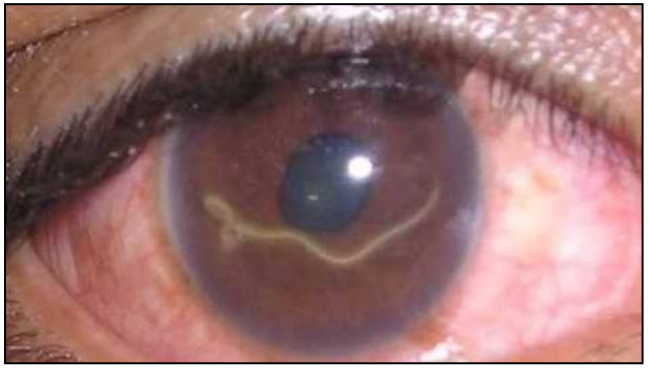
- Uncommon in the developed world.
- Usually have **complex life cycles**.
  - **Helminths**
  - Arthropods (e.g. scabies, pediculosis)

6. **Prion**

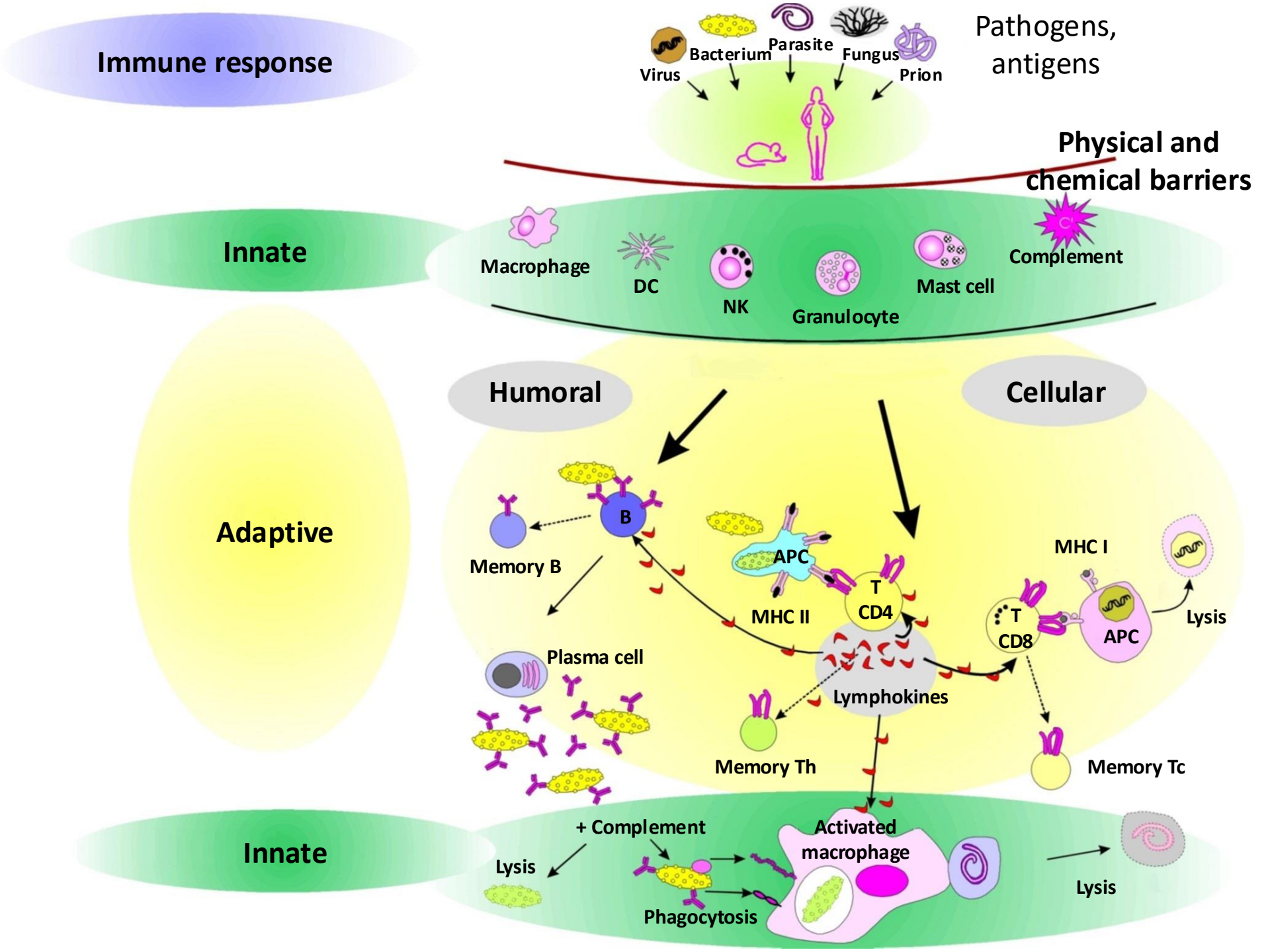
Infectious **protein** (PrP) with abnormal folding.

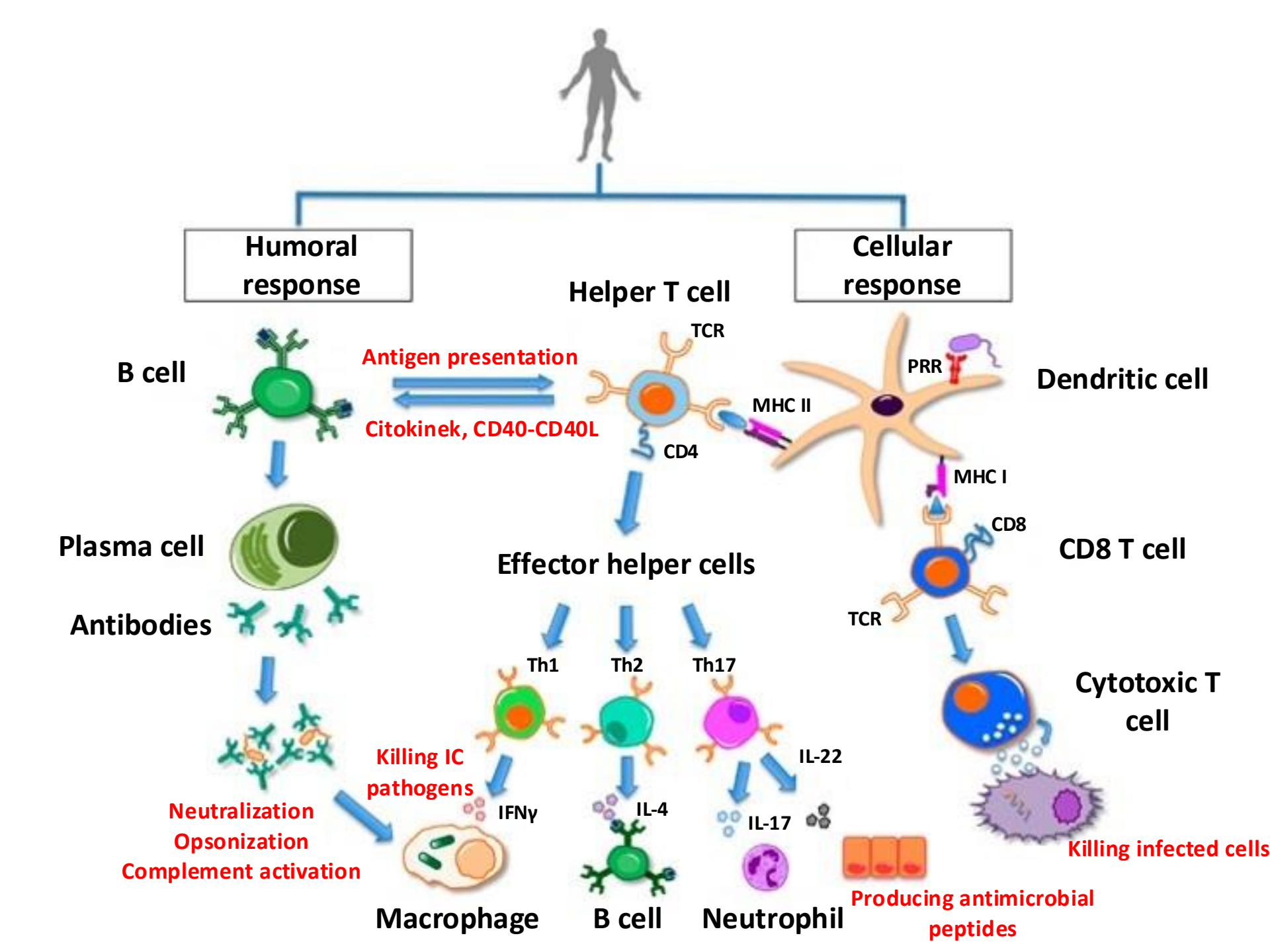
Causes different types of TSE.[8.]

(TSE: Transmissible spongiform encephalopathy)

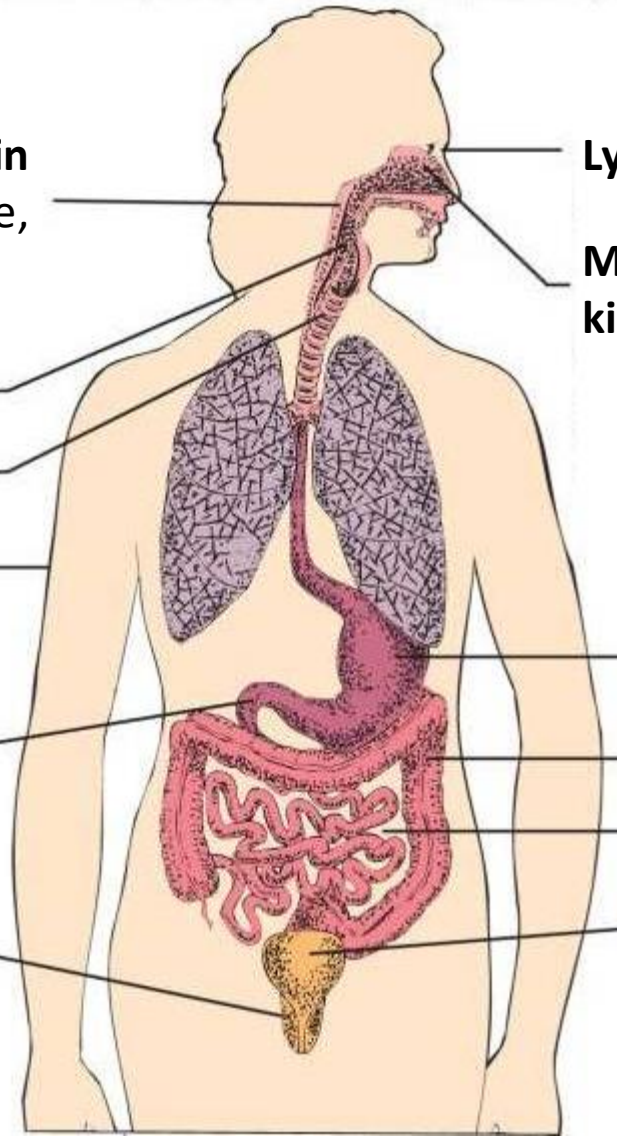


Loa loa („eye worm") infection of the conjuctiva. (Approx. 10 million infected people live in Africa.[10.])


Immune response
Pathogens, antigens
Virus
Bacterium
Parasite
Fungus
Prion
Physical and chemical barriers
Innate
Macrophage
DC
NK
Granulocyte
Mast cell
Complement
Adaptive
Humoral
Cellular
B
Memory B
Plasma cell
APC
MHC II
T CD4
Lymphokines
Memory Th
MHC I
T CD8
APC
Lysis
Memory Tc
Innate
+ Complement
Lysis
Phagocytosis
Activated macrophage
Lysis


Humoral response
Helper T cell
Cellular response
B cell
Antigen presentation
Citokinek, CD40-CD40L
TCR
CD4
MHC II
PRR
Dendritic cell
MHC I
CD8
CD8 T cell
TCR
Plasma cell
Antibodies
Effector helper cells
Th1
Th2
Th17
Cytotoxic T cell
Killing IC pathogens
IFNγ
IL-4
IL-22
IL-17
Neutralization
Opsonization
Complement activation
Killing infected cells
Producing antimicrobial peptides
Macrophage
B cell
Neutrophil

Physical and chemical barriers



Antimicrobial agents in saliva (lysozyme, peroxidase, lactoferrin, etc.)

Mucus**, resident flora**

Mucus, kinocilia

Keratinocytes, acidic pH, resident flora

Rapid pH change

Acidic pH, **resident flora of the vagina**

Lysozyme in tears

Mucus, removal of particles by **kinocilia** towards the pharynx

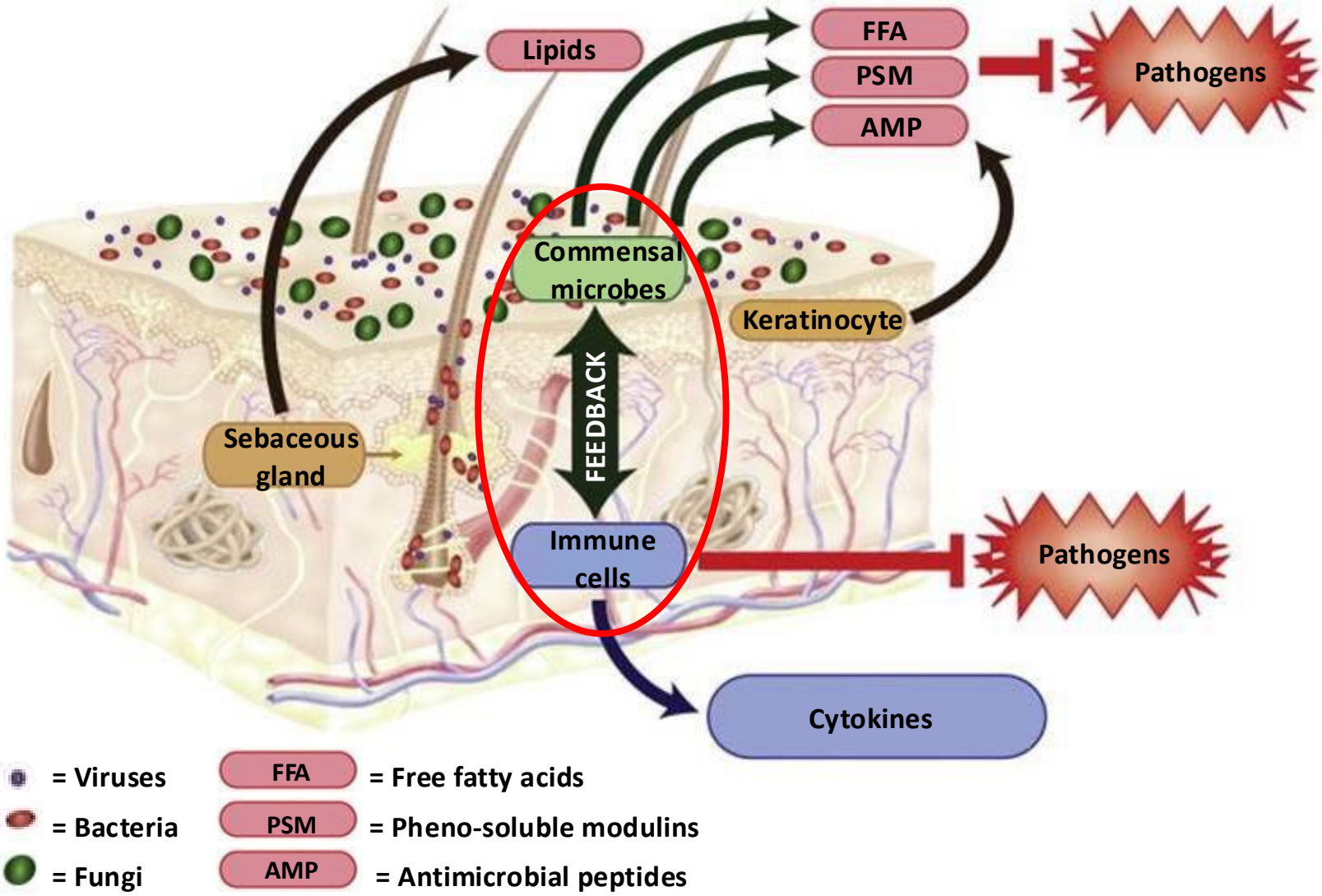
Mucus, **gastric acid**

Mucus, **resident flora**

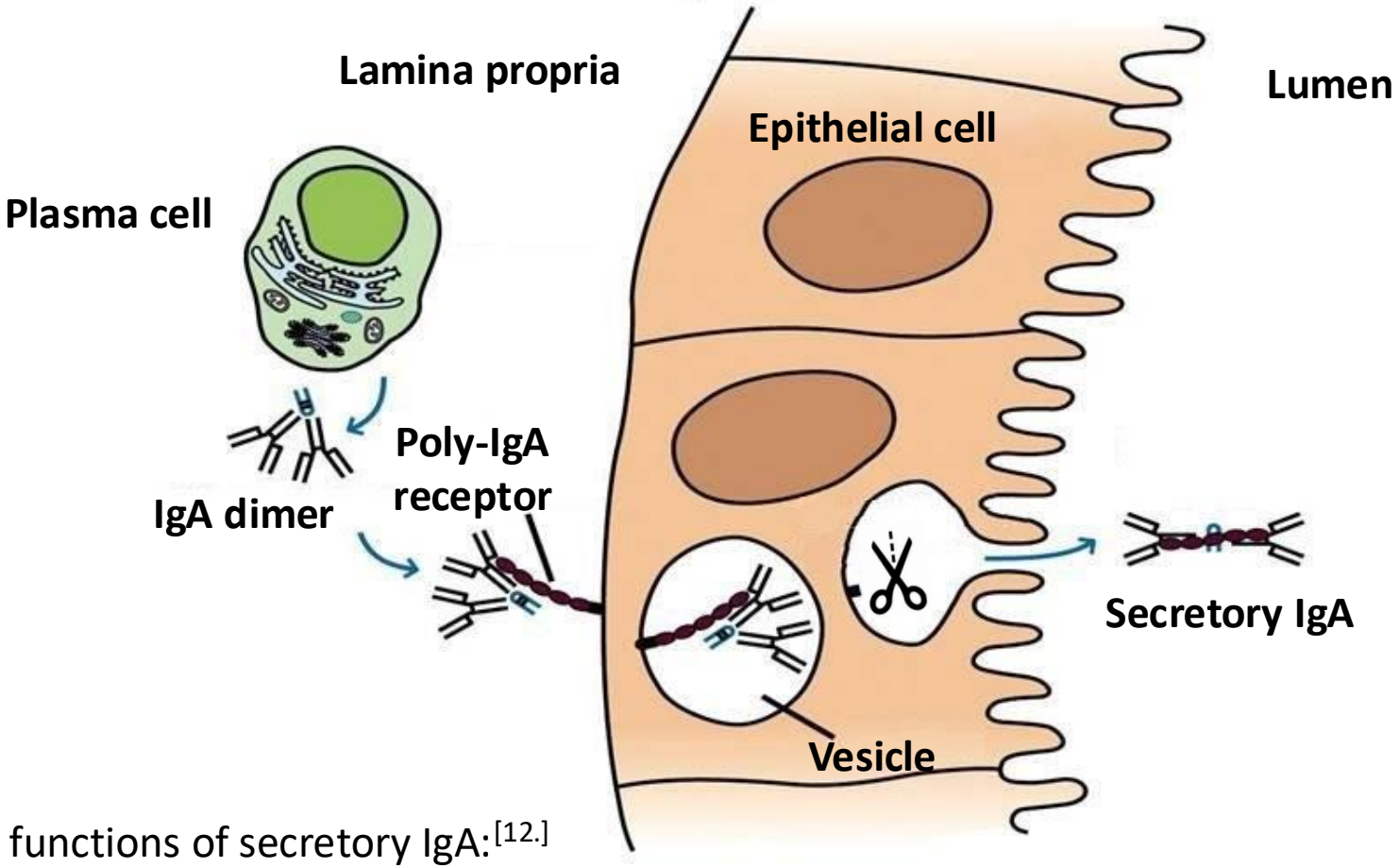
Mucus, peristalsis

Flushing of urinary tract

Role of the skin microbiome[11.]



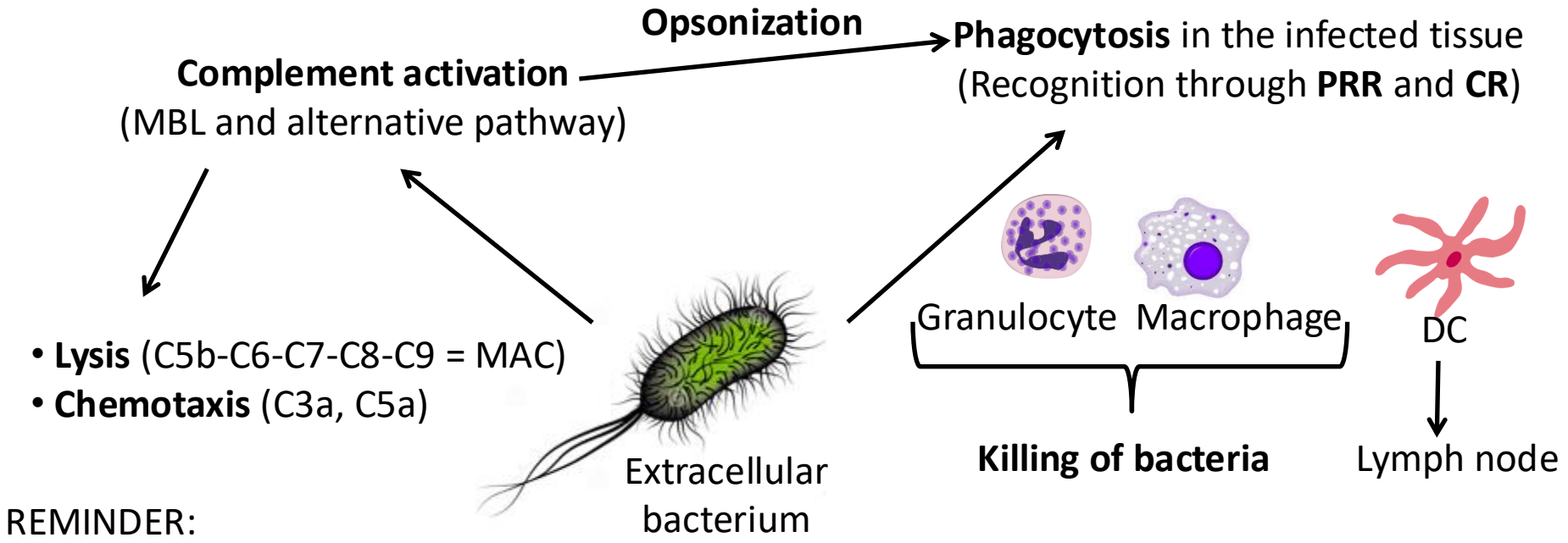
Mechanism of IgA secretion



Main functions of secretory IgA:[12.]

- **Neutralization and agglutination** of pathogens
- Retrograde transport of antigens (in the form of immunocomplexes from the lumen to the lamina propria)

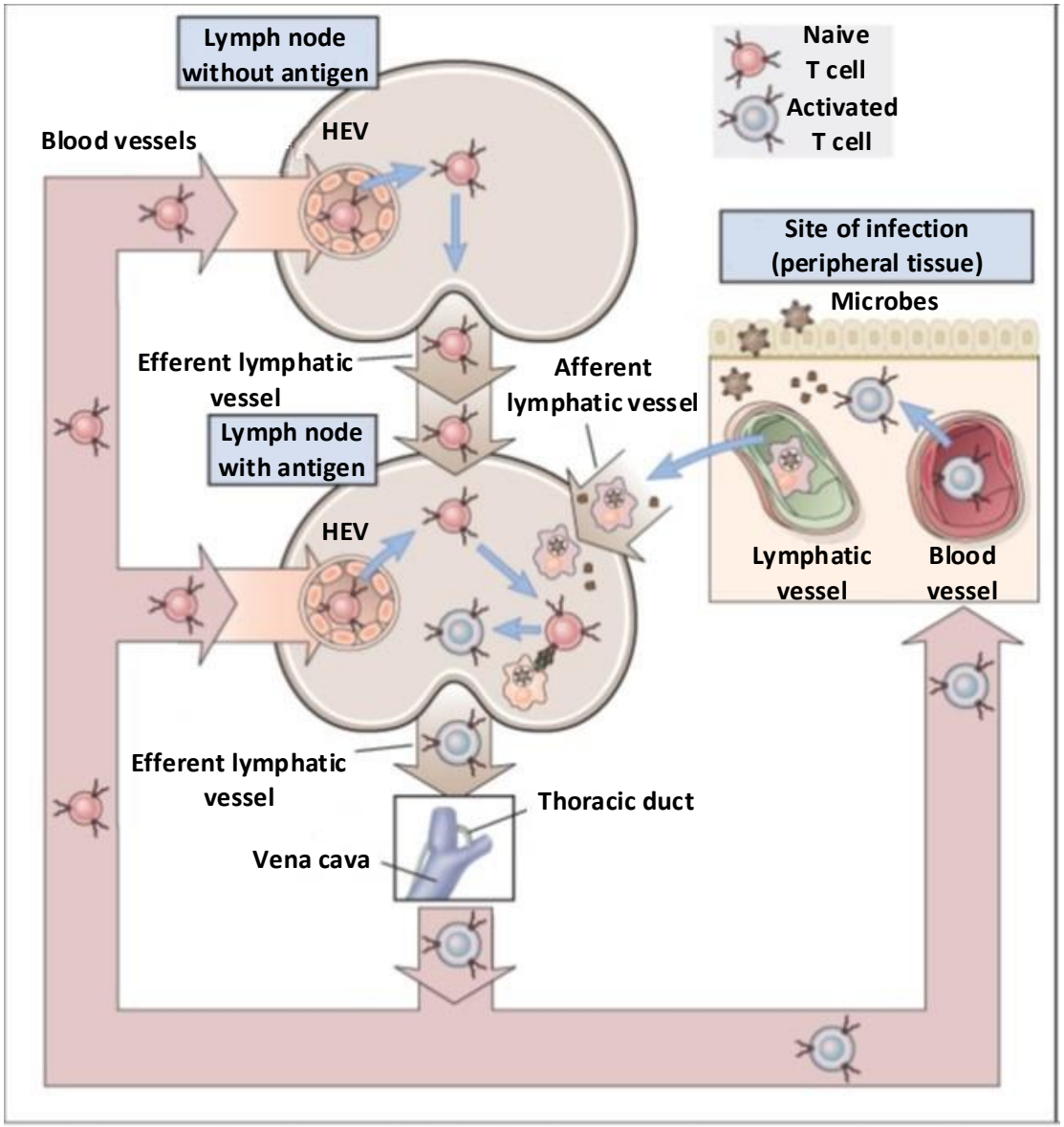
Innate immune response against extracellular bacteria



REMINDER:

	Innate	Adaptive
Recognition	**Pattern-based (not antigen-specific)**	**Antigen-specific**
Kinetics	**Quick (minutes, hours)**	**Slow (days, weeks)**
Amplification of response	**Linear**	**Exponential**
Immunological memory	**No**	**Yes**

Filtration of lymph by nodes



The antigens of the microbes will reach the draining lymph node in different forms recognized by different cells:

- **Native form** (e.g. the entire microbe or soluble native antigens derived from dead microbes)

↓

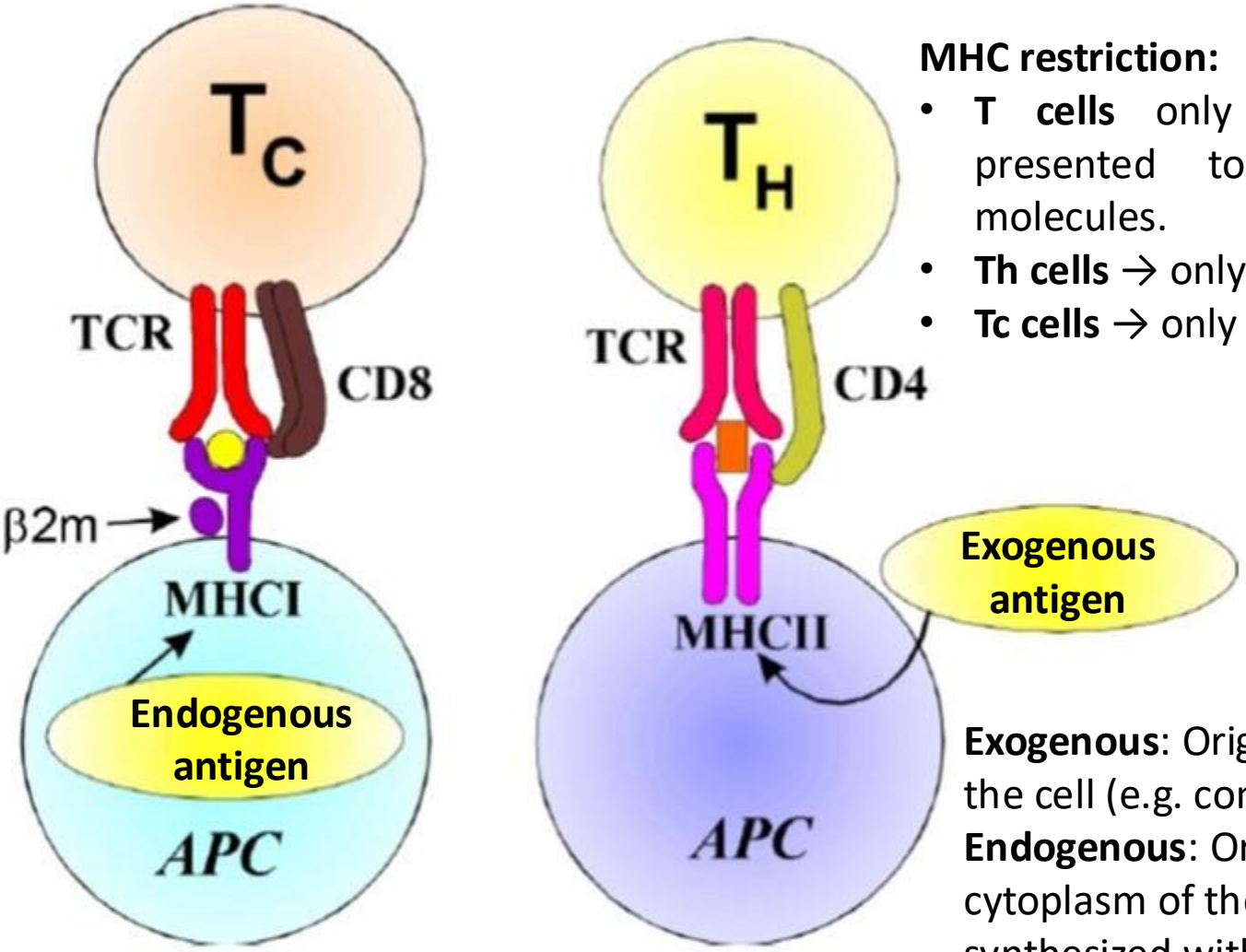
Recognized by **B cells**

- In a **processed form** presented by dendritic cells:

↓

Recognized by CD4+ **T helper** cells

Antigen recognition of T cells

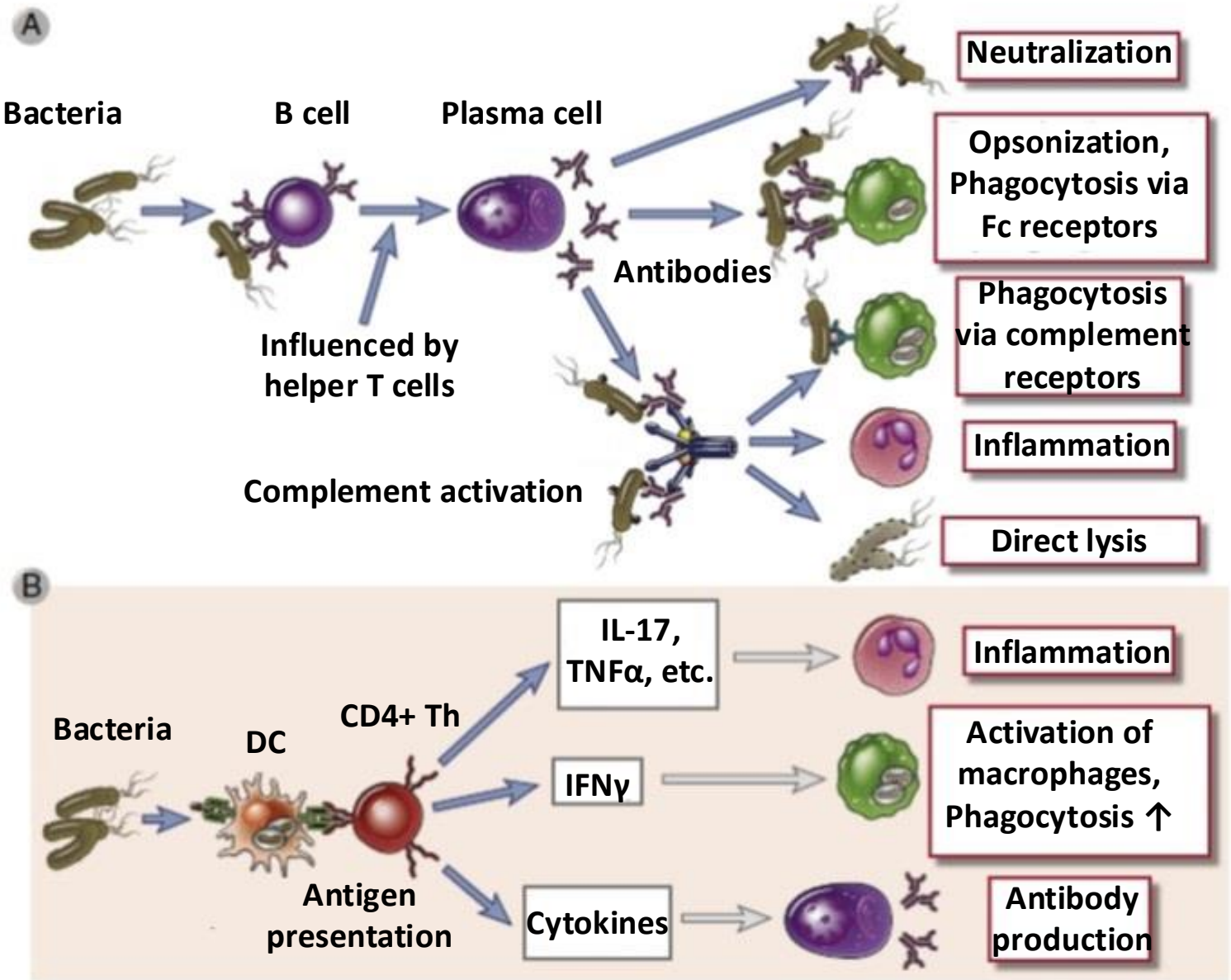


MHC restriction:

- **T cells** only recognize antigens presented to them via **MHC** molecules.
- **Th cells** → only via **MHC II**
- **Tc cells** → only via **MHC I**

Exogenous: Originates from outside the cell (e.g. components of bacteria)
Endogenous: Originates from the cytoplasm of the cell (e.g. viral proteins synthesized within the infected cells)

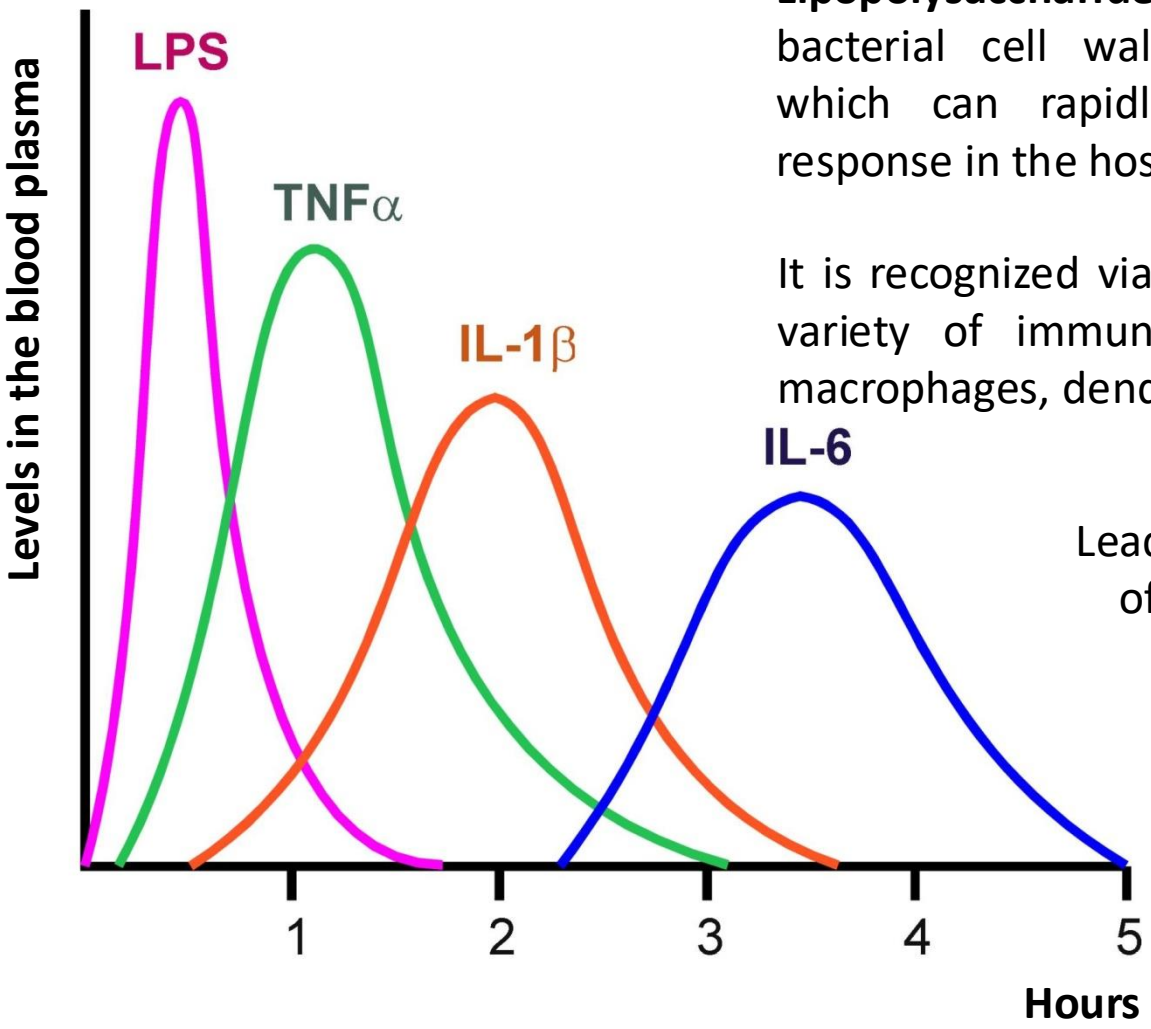
Adaptive response against EC bacteria



Possible complications of immune responses against EC bacteria

- The mechanisms involved in the defense against pathogens (acute phase proteins, inflammatory cytokines, reactive oxygen species, etc.) are normally under control.
- Dysregulation of the immune response can lead to:
  - **Insufficient response** (e.g. immunodeficiency): Dissemination of the infection
  - **Over activation**: Tissue damage, cytokine storm, circulatory shock
- In people who are genetically susceptible to such conditions the immune response to certain pathogens **can lead to autoimmunity** (see later), e.g.:
  - *Streptococcus pyogenes* → Rheumatic fever, glomerulonephritis[13.]
  - *Campylobacter jejuni* → Guillain-Barré syndrome (autoimmune peripheral neuropathy)[14.]

Levels of cytokines in the blood after Gram-negative infections



Lipopolysaccharide (LPS) is a component of the bacterial cell wall in Gram-negative bacteria which can rapidly cause a strong immune response in the host (it is a bacterial endotoxin)

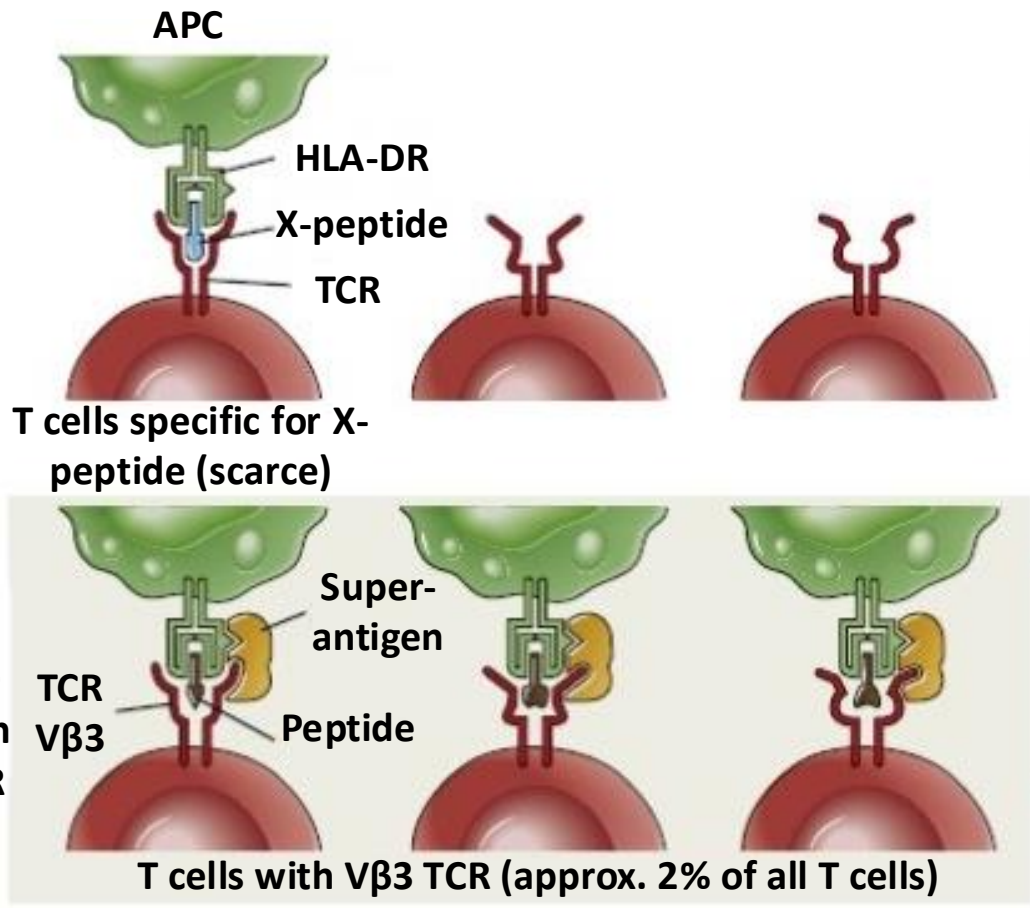
↓

It is recognized via **TLR4** and **CD14**. (Found on a variety of immune cells such as monocytes, macrophages, dendritic cells, B cells, etc.)

↓

Leads to the intense production of **inflammatory cytokines**.

Superantigens



Some pathogens (such as *Staphylococcus aureus* bacteria) produce toxins (superantigens) that can **activate many T cells** in a **non-antigen-specific w**ay (possibly 20% of all T cells simultaneously[15.]). These cells will produce inflammatory cytokines in large amounts that will lead to circulatory shock. (Toxic shock syndrome[16.])

Intracellular bacteria

Some bacteria reside in the infected cells and evade the humoral components of the immune response. (e.g. complement, antibodies)

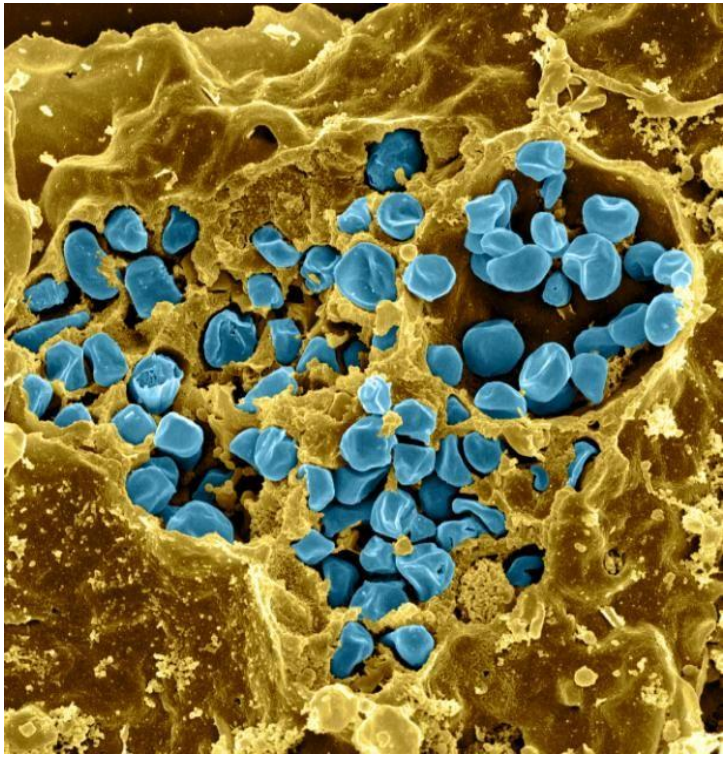
↓

The **Th1**-induced **cellular immunity** can combat them[17, 18.]

Problem: Some of them **even survive in phagocytes**.[19.] They apply different strategies to survive in these cells (see later in microbiology):

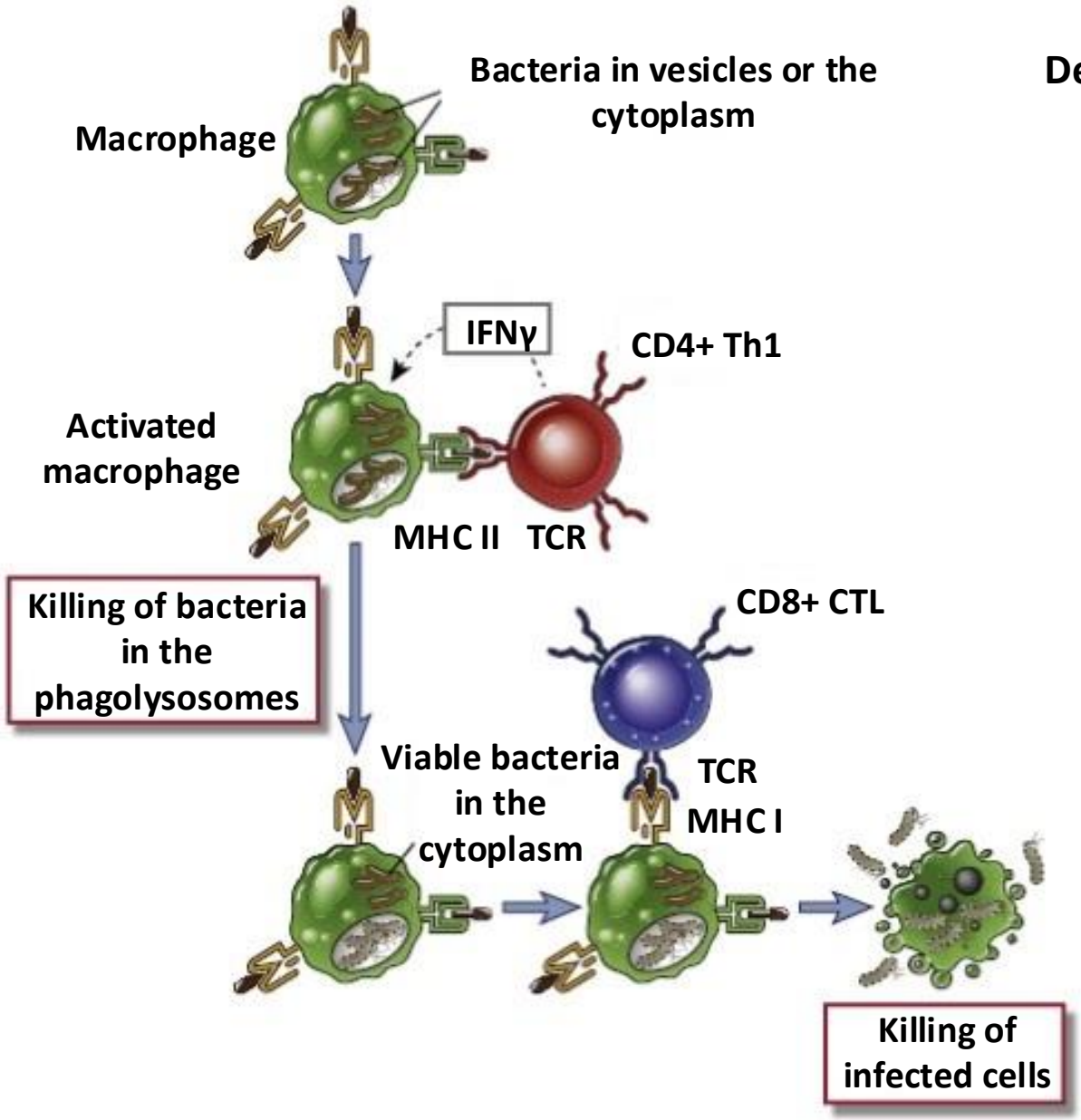
- **Escape** from the vesicles **to the cytoplasm** (e.g. *Shigella, Listeria, Francisella*)[20, 21.]
- **Inhibit the maturation of phagolysosomes** (e.g. *Mycobacterium, Legionella*)[22.]
- **Even survive in the phagolysosomes** (e.g. *Coxiella burnetii, Yersinia*)[23.]

These bacteria can induce a **chronic cellular response** that also causes damage to nearby tissues (see: Type IV. hypersensitivity, e.g. in the case of tuberculosis)



Francisella tularensis bacteria in a murine macrophage. Some cells can be seen in vesicles others are located in the cytoplasm. (Scanning electron microscopy)

Immune response against IC bacteria



Detection of IC bacteria with PRRs

↓

Production of **IL-12**

↓

Antigen presentation to CD4+ helper cells

↓

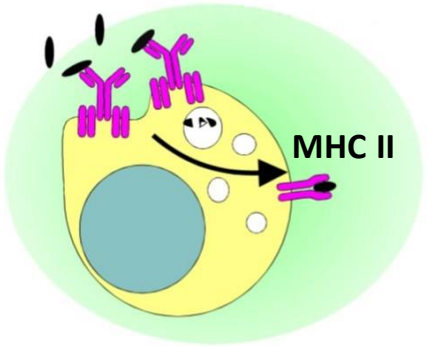
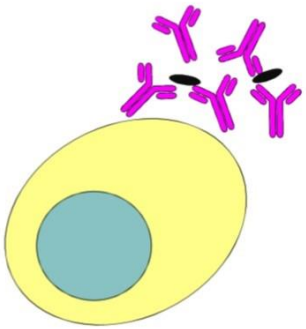
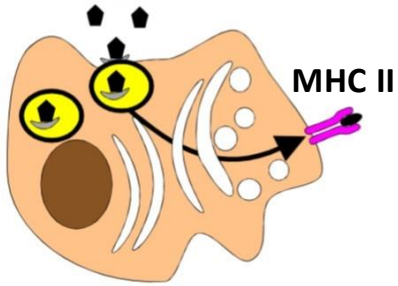
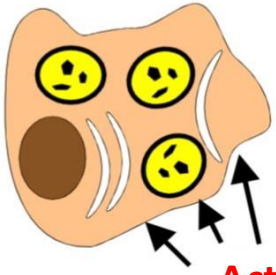
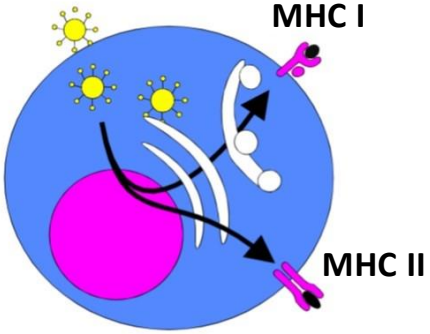
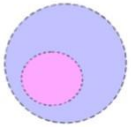
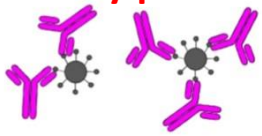
Production of **IFNγ**

↓

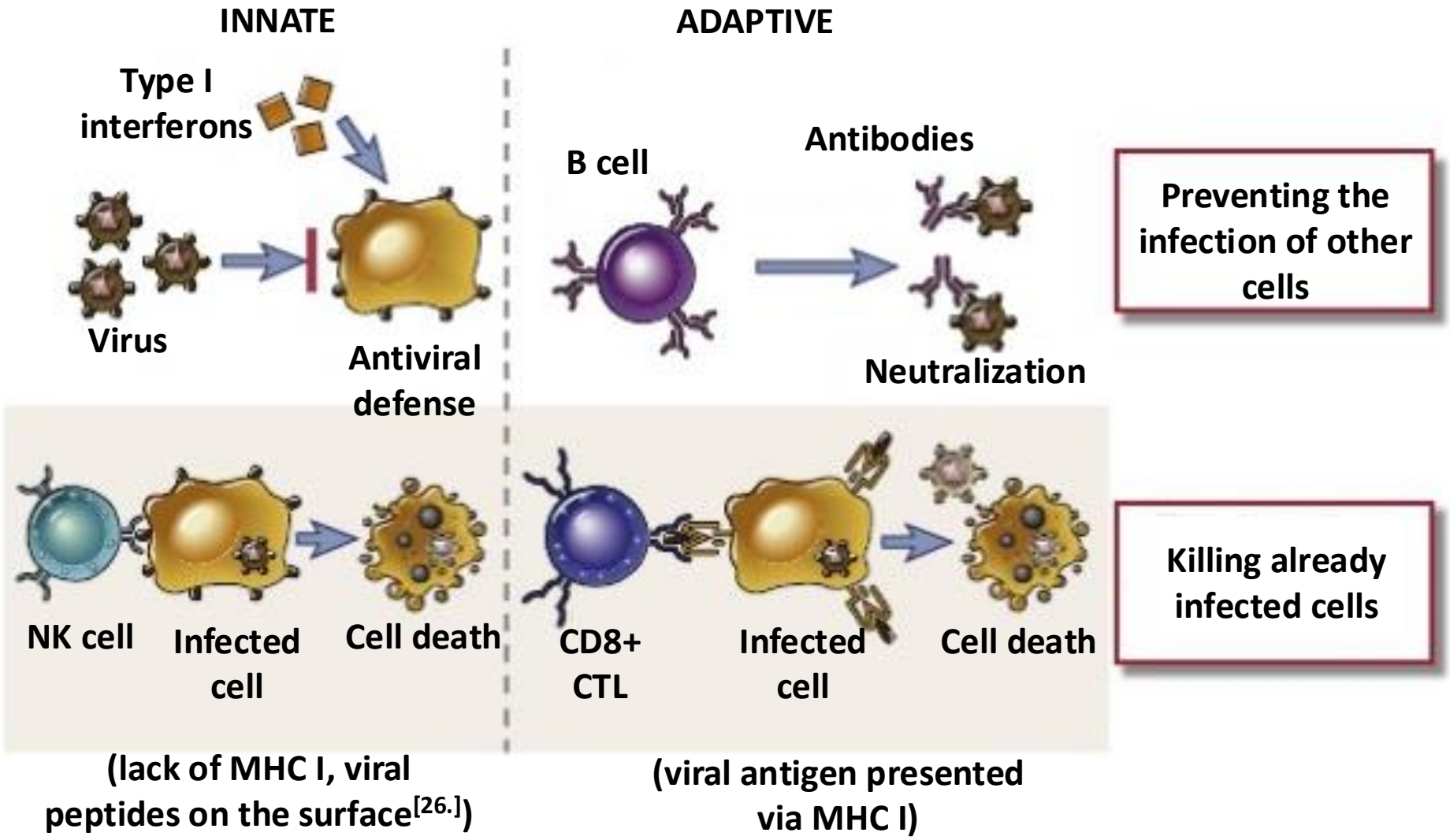
Activation of macrophages

↓

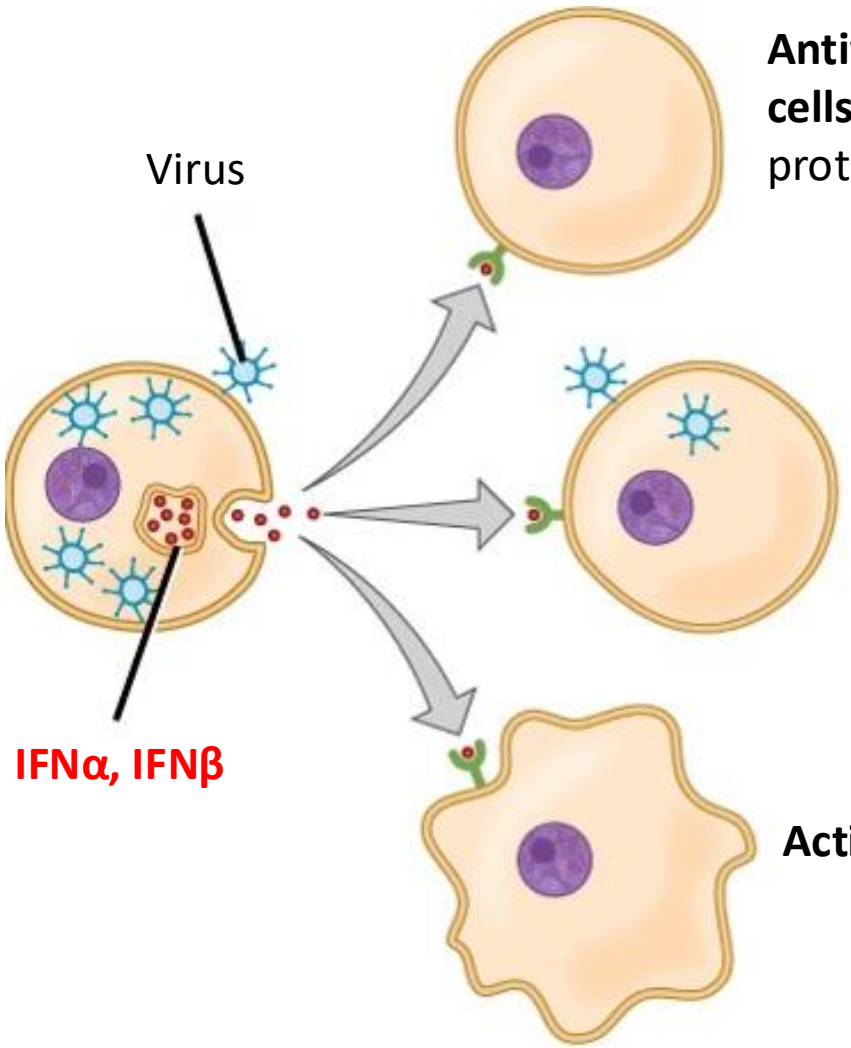
Killing of remaining infected cells by **CTL** and **NK cells**

Type of pathogen	Antigen presentation and processing	Response
Extracellular 	Degradation: **In acidic vesicles** Binding of peptides: **MHC II** Presentation: **To CD4+ T cells**	**Antibody production** 
Intravesicular 	Degradation: **In acidic vesicles** Binding of peptides: **MHC II** Presentation: **To CD4+ T cells**	**Killing of pathogen in vesicles**  **Activation by Th1 cells**
Cytosolic 	Degradation: **In the cytoplasm** Binding of peptides: **MHC I, MHC II** Presentation: **To CD8+ T cells, To CD4+ T cells**	**Killing the infected cell**  **Antibody production** 

Immune response against viruses[24, 25.]



Type I („natural”) interferons[27.]

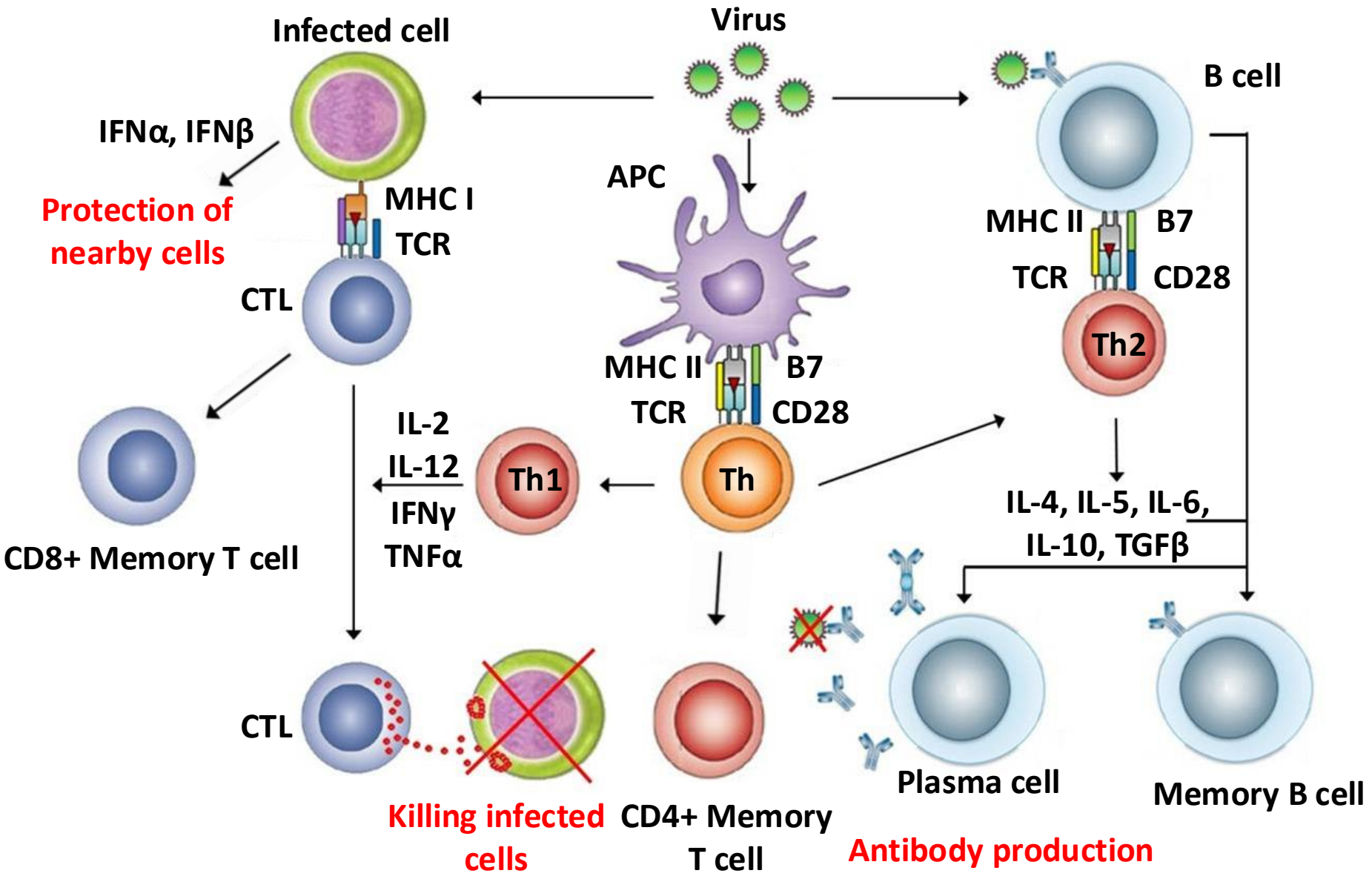


Antiviral protection of nearby uninfected cells (e.g. degradation of RNA, reducing protein synthesis, etc.)

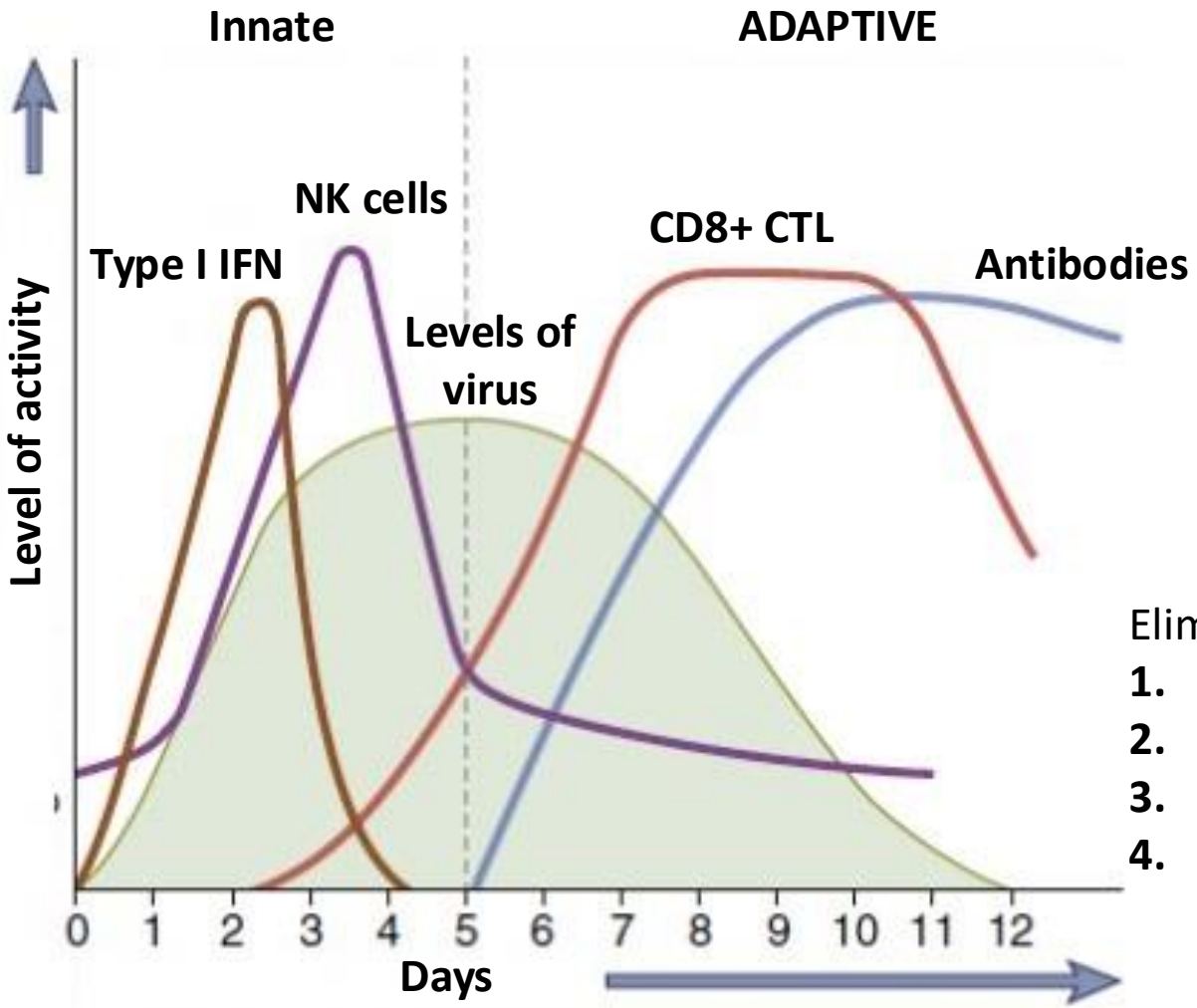
Apoptosis of nearby infected cells

Activation of immune cells

Adaptive response against viruses



Activation of the immune response after viral infection



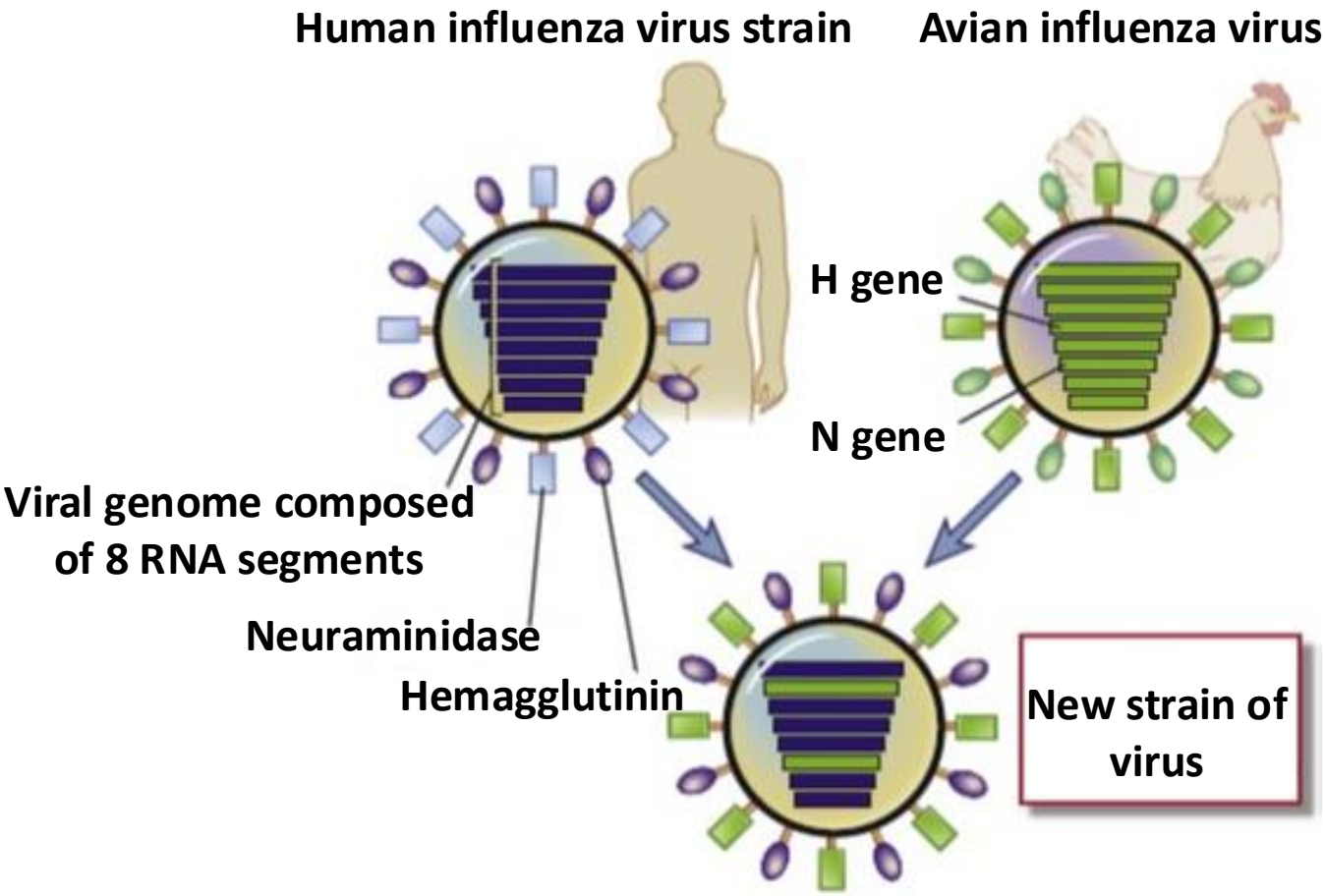
Elimination of infected cells:

1. **Complement-mediated lysis**
2. **CD8+ cytotoxic T cells**
3. **NK cells via KAR**
4. **ADCC**

Problems

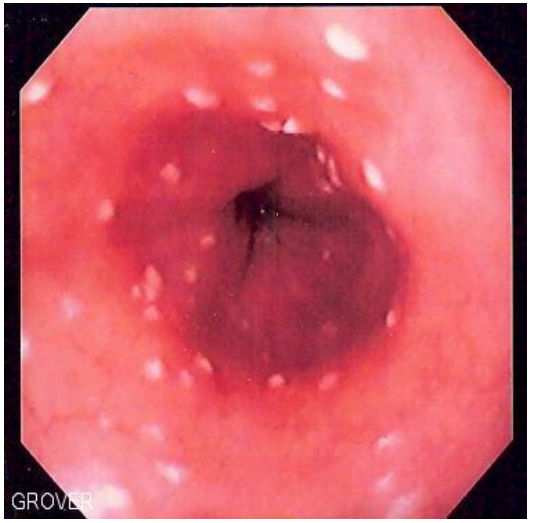
- Viruses have several ways of evading the immune response, such as:
  - **Fast mutation rate** that leads constantly changing their antigens (characteristic for **RNA viruses**, e.g. HIV[28.], influenza[29.] and rhinoviruses[30.])
  - **Antigenic shift** (e.g. influenza)
  - Blocking of antigen presentation (e.g. EBV[31.])
  - Killing adaptive immune cells (e.g. HIV[32.])
  - Expression of viral MHC I-like molecules on infected cells (evades killing of infected cell by NK cells, e.g. CMV[33.])
  - Preventing recognition via PRRs (e.g. Ebola viruses[34.])
  - Inhibition of type I interferons (e.g. Ebola viruses[34.])
  - Many viruses evade the immune response by residing in infected cells in a **latent form** and only reactivate to certain (usually unknown) trigger effects. (e.g. herpes viruses[35.])
- Because the above mechanisms some viruses cause **chronic infections** that **persist throughout the entire life of the host** and if the immune system weakens for some reason **they can reactivate**.[35.]

Phenomenon of antigenic shift



Immune response against fungi

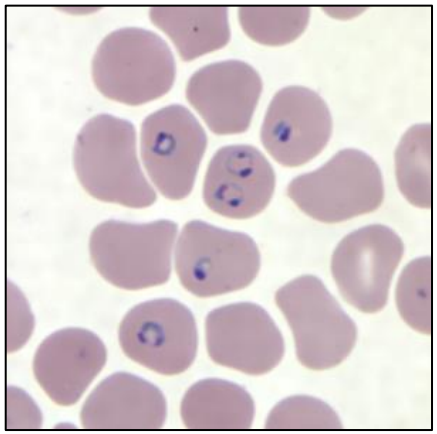
- **Much less is known** about the immune response against fungi compared to other pathogens.[3.] (They are mainly restricted to patients with **immunodeficiencies.**)
- Some of the pathogens are extracellular, others are intracellular:
  - **EC fungi** → Trigger **humoral immune responses**
  - **IC fungi** → Trigger **cellular immune responses**
- Innate recognition: Cells recognize fungal PAMPs (e.g. β-glucan, chitin, mannan) via **PRRs** → **Phagocytosis**, mainly by **macrophages** and **neutrophils** (neutropenia can lead to severe fungal infections, see later in your clinical studies)
- The fungal cell wall can activate the **complement system**.[36.] (mainly through the MBL pathway, see the lectures for details)



Opportunistic *Candida* infection of the esophagus in a patient receiving chemotherapy. (endoscopic image)

Immune response against unicellular parasites

- **One of the most significant group** of pathogens. (198 million cases of Malaria alone in 2013 worldwide which turned out to be lethal in 584.000 cases[37.])
- Most of them have complex life cycles, different mechanisms could be effective against the different forms of the same pathogen.
- **Intracellular protozoa** → **Cellular immunity** (phagocytes, NK cells, CD8+ T cells)
- **Extracellular protozoa** → **Humoral immunity** (complement, antibodies)
- Those that have both extracellular and intracellular forms trigger both. (e.g. *Plasmodium*)
- Some IC parasites can **also survive within macrophages** (e.g. *Leishmania*), which makes the activation of macrophages via the production of **IFNγ** by **Th1 cells** necessary and leads to a **chronic response** that also **damages the tissues**.[38.] (Type IV. hypersensitivity)



Trophozoites of *Plasmodium falciparum* in red blood cells in a patient with Malaria.

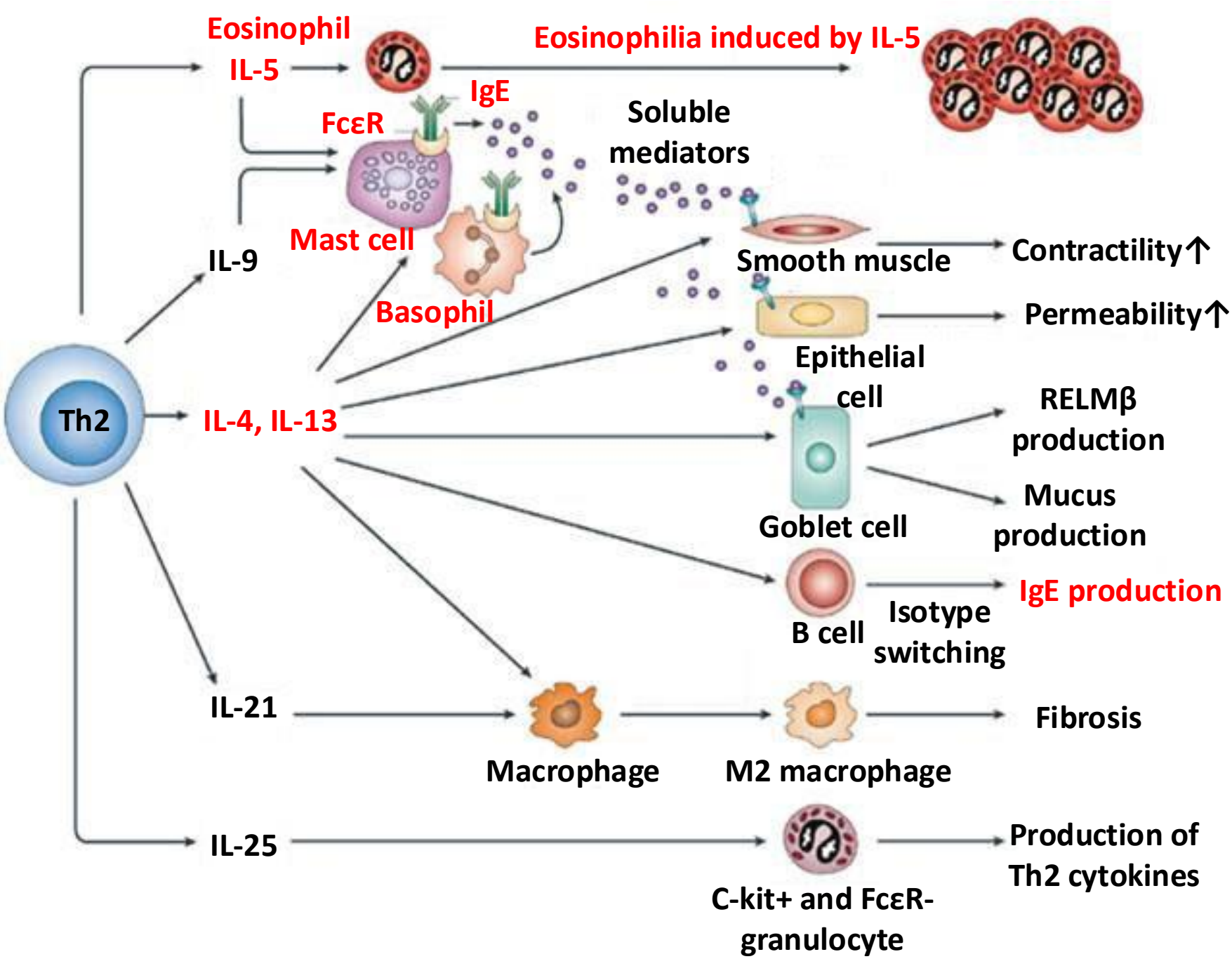
Immune response against multicellular parasites

- They also have complex life cycles.
- The cells and mechanisms that participate in the defense are different from those previously mentioned[39.], such as.:
  - **Eosinophil granulocytes** (degranulation → 2th practice)
  - **Mast cells, basophil granulocytes**
  - **IL-4, IL-5** and **IL-13** are the dominant cytokines → **IgE production**, eosinophil counts↑
- The permeability and the contractility of the intestines both have a major role in the defense against intestinal worms.
- Problems:
  - Many of them reside in places **inaccessible for the immune system.** (e.g. intestinal helminths in the intestinal lumen)
  - **Their integuments protect them** even against large numbers of immune cells.

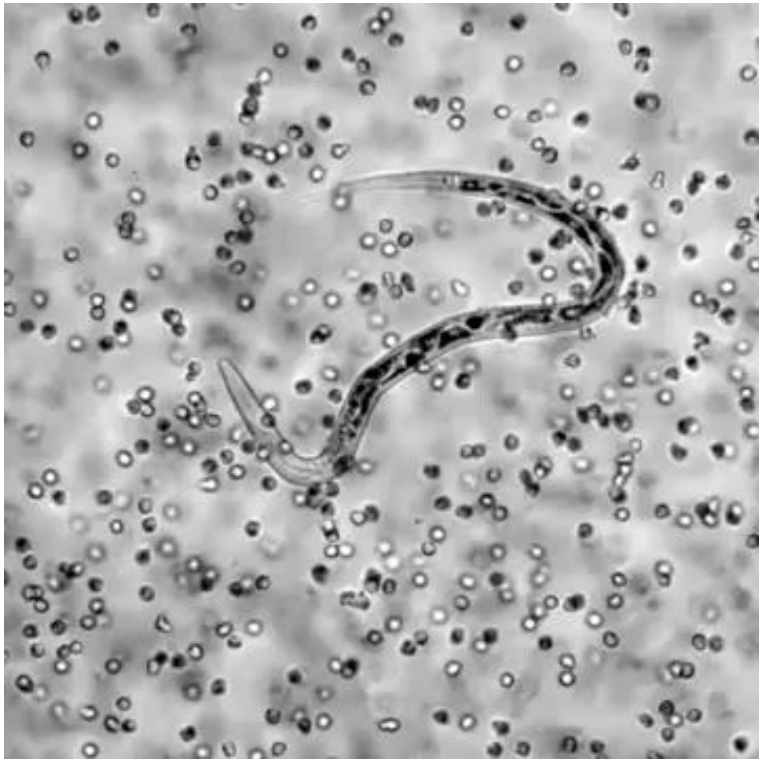
↓

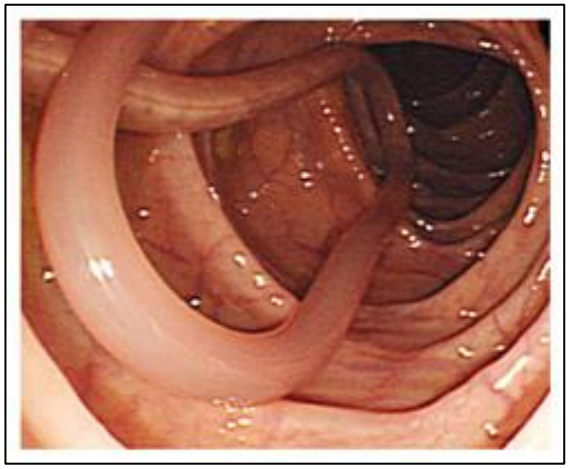
Most of them cause **chronic infections** and the host cannot get rid of them without **medical help**.

It is estimated that roughly 1,2 BILLION people are infected with *Ascaris*![40.]


Eosinophil
IL-5
Eosinophilia induced by IL-5
IgE
FcεR
Soluble
mediators
Mast cell
IL-9
Basophil
Smooth muscle
Contractility↑
Permeability↑
Epithelial
cell
Th2
IL-4, IL-13
RELMβ
production
Mucus
production
Goblet cell
B cell
IgE production
Isotype
switching
IL-21
Macrophage
M2 macrophage
Fibrosis
IL-25
Production of
Th2 cytokines
C-kit+ and FcεR-
granulocyte

Thank you for your attention!





Ascaris lumbricoides in a human intestine. (endoscopic image)

Video: Human eosinophil granulocytes surround a *C. elegans* larva.

References 1.

1. Human Microbiome Project Consortium: **A framework for human microbiome research.** *Nature.* 2012 Jun 13;486(7402):215-21. doi: 10.1038/nature11209.
2. Bianconi E[1], et al.: **An estimation of the number of cells in the human body.** *Ann Hum Biol.* 2013 Nov-Dec;40(6):463-71. doi: 10.3109/03014460.2013.807878. Epub 2013 Jul 5.
3. Romani L[1]: **Immunity to fungal infections.** *Nat Rev Immunol.* 2011 Apr;11(4):275-88. doi: 10.1038/nri2939. Epub 2011 Mar 11.
4. Sokulska M[1], et al.: **Pneumocystis jirovecii--from a commensal to pathogen: clinical and diagnostic review.** *Parasitol Res.* 2015 Oct;114(10):3577-85. doi: 10.1007/s00436-015-4678-6. Epub 2015 Aug 19.
5. White NJ[1], et al.: **Malaria.** *Lancet.* 2014 Feb 22;383(9918):723-35. doi: 10.1016/S0140-6736(13)60024-0. Epub 2013 Aug 15.
6. Kissinger P[1]: **Trichomonas vaginalis: a review of epidemiologic, clinical and treatment issues.** *BMC Infect Dis.* 2015 Aug 5;15:307. doi: 10.1186/s12879-015-1055-0.
7. Montoya JG[1], Liesenfeld O: **Toxoplasmosis.** *Lancet.* 2004 Jun 12;363(9425):1965-76.
8. Aguzzi A[1], Nuvolone M, Zhu C: **The immunobiology of prion diseases.** *Nat Rev Immunol.* 2013 Dec;13(12):888-902. doi: 10.1038/nri3553. Epub 2013 Nov 5.
9. Poole DN[1], McClelland RS: **Global epidemiology of Trichomonas vaginalis.** *Sex Transm Infect.* 2013 Sep;89(6):418-22. doi: 10.1136/sextrans-2013-051075. Epub 2013 Jun 6.
10. Metzger WG[1], Mordmüller B[2]: **Loa loa-does it deserve to be neglected?** *Lancet Infect Dis.* 2014 Apr;14(4):353-7. doi: 10.1016/S1473-3099(13)70263-9. Epub 2013 Dec 12.
11. Zeeuwen PL[1], et al.: **Microbiome and skin diseases.** *Curr Opin Allergy Clin Immunol.* 2013 Oct;13(5):514-20. doi: 10.1097/ACI.0b013e328364ebeb.
12. Mantis NJ[1], Rol N, Corthésy B: **Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut.** *Mucosal Immunol.* 2011 Nov;4(6):603-11. doi: 10.1038/mi.2011.41. Epub 2011 Oct 5.

References 2.

13. Cunningham MW[1]: **Rheumatic fever, autoimmunity, and molecular mimicry: the streptococcal connection.** *Int Rev Immunol.* 2014 Jul-Aug;33(4):314-29. doi: 10.3109/08830185.2014.917411. Epub 2014 Jun 3.
14. Yuki N[1], Odaka M: **Ganglioside mimicry as a cause of Guillain-Barré syndrome.** *Curr Opin Neurol.* 2005 Oct;18(5):557-61.
15. Stach CS[1], Herrera A, Schlievert PM: **Staphylococcal superantigens interact with multiple host receptors to cause serious diseases.** *Immunol Res.* 2014 Aug;59(1-3):177-81. doi: 10.1007/s12026-014-8539-7.
16. Kulhankova K[1], King J, Salgado-Pabón W: **Staphylococcal toxic shock syndrome: superantigen-mediated enhancement of endotoxin shock and adaptive immune suppression.** *Immunol Res.* 2014 Aug;59(1-3):182-7. doi: 10.1007/s12026-014-8538-8.
17. Kaufmann SH[1]: **Immunity to intracellular bacteria.** *Annu Rev Immunol.* 1993;11:129-63.
18. Weiss G[1], Schaible UE: **Macrophage defense mechanisms against intracellular bacteria.** *Immunol Rev.* 2015 Mar;264(1):182-203. doi: 10.1111/imr.12266.
19. Ernst RK[1], Guina T, Miller SI: **How intracellular bacteria survive: surface modifications that promote resistance to host innate immune responses.** *J Infect Dis.* 1999 Mar;179 Suppl 2:S326-30.
20. Freitag NE[1], Port GC, Miner MD: **Listeria monocytogenes - from saprophyte to intracellular pathogen.** *Nat Rev Microbiol.* 2009 Sep;7(9):623-8. doi: 10.1038/nrmicro2171. Epub 2009 Aug 3.
21. Chong A[1], Celli J: **The francisella intracellular life cycle: toward molecular mechanisms of intracellular survival and proliferation.** *Front Microbiol.* 2010 Dec 28;1:138. doi: 10.3389/fmicb.2010.00138. eCollection 2010.
22. Rohde K[1], Yates RM, Purdy GE, Russell DG: **Mycobacterium tuberculosis and the environment within the phagosome.** *Immunol Rev.* 2007 Oct;219:37-54.
23. Voth DE[1], Heinzen RA: **Lounging in a lysosome: the intracellular lifestyle of Coxiella burnetii.** *Cell Microbiol.* 2007 Apr;9(4):829-40.

References 3.

24. Takeuchi O[1], Akira S: **Innate immunity to virus infection.** *Immunol Rev.* 2009 Jan;227(1):75-86. doi: 10.1111/j.1600-065X.2008.00737.x.
25. Aoshi T[1], et al.: **Innate and adaptive immune responses to viral infection and vaccination.** *Curr Opin Virol.* 2011 Oct;1(4):226-32. doi: 10.1016/j.coviro.2011.07.002. Epub 2011 Jul 30.
26. Lanier LL[1]: **Evolutionary struggles between NK cells and viruses.** *Nat Rev Immunol.* 2008 Apr;8(4):259-68. doi: 10.1038/nri2276. Epub 2008 Mar 14.
27. McNab F[1], et al.: **Type I interferons in infectious disease.** *Nat Rev Immunol.* 2015 Feb;15(2):87-103. doi: 10.1038/nri3787.
28. Alter G[1], Ackerman ME[2]: **An antibody tag-team: driving neutralization through escape.** *Trends Immunol.* 2014 Sep;35(9):403-5. doi: 10.1016/j.it.2014.07.009. Epub 2014 Aug 15.
29. van de Sandt CE[1], Kreijtz JH, Rimmelzwaan GF: **Evasion of influenza A viruses from innate and adaptive immune responses.** *Viruses.* 2012 Sep;4(9):1438-76. doi: 10.3390/v4091438. Epub 2012 Sep 3.
30. Kirchberger S[1], Majdic O, Stockl J: **Modulation of the immune system by human rhinoviruses.** *Int Arch Allergy Immunol.* 2007;142(1):1-10. Epub 2006 Sep 29.
31. Hu Z[1], Usherwood EJ: **Immune escape of γ-herpesviruses from adaptive immunity.** *Rev Med Virol.* 2014 Nov;24(6):365-78. doi: 10.1002/rmv.1791. Epub 2014 Apr 15.
32. Douek DC[1], et al.: **HIV preferentially infects HIV-specific CD4+ T cells.** *Nature.* 2002 May 2;417(6884):95-8.
33. Pyzik M[1], et al.: **Viral MHC class I-like molecule allows evasion of NK cell effector responses in vivo.** *J Immunol.* 2014 Dec 15;193(12):6061-9. doi: 10.4049/jimmunol.1401386. Epub 2014 Nov 12. [Erratum in: *J Immunol.* 2015 Jun 15;194(12):6192.]
34. Misasi J[1], Sullivan NJ[2]: **Camouflage and misdirection: the full-on assault of ebola virus disease.** *Cell.* 2014 Oct 23;159(3):477-86. doi: 10.1016/j.cell.2014.10.006. Epub 2014 Oct 16.

References 4.

35. Sinclair JH[1], Reeves MB: **Human cytomegalovirus manipulation of latently infected cells.** *Viruses.* 2013 Nov 21;5(11):2803-24. doi: 10.3390/v5112803.
36. Speth C[1], et al.: **Complement and fungal pathogens: an update.** *Mycoses.* 2008 Nov;51(6):477-96. doi: 10.1111/j.1439-0507.2008.01597.x. Epub 2008 Aug 13.
37. WHO: **Fact sheet on the World Malaria Report 2014** (http://www.who.int/malaria/media/world_malaria_report_2014/en/)
38. Arango Duque G[1], Descoteaux A[2]: **Leishmania survival in the macrophage: where the ends justify the means.** *Curr Opin Microbiol.* 2015 Aug;26:32-40. doi: 10.1016/j.mib.2015.04.007. Epub 2015 May 17.
39. Anthony RM[1], et al.: **Protective immune mechanisms in helminth infection.** *Nat Rev Immunol.* 2007 Dec;7(12):975-87.
40. Dold C[1], Holland CV: **Ascaris and ascariasis.** *Microbes Infect.* 2011 Jul;13(7):632-7. doi: 10.1016/j.micinf.2010.09.012. Epub 2010 Oct 8.