Basic Immunology

Effector mechanisms of cell-mediated immune responses (CMI):

Cytotoxicity

Th1 cell-mediated macrophage activation

Lecture 17-18

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.

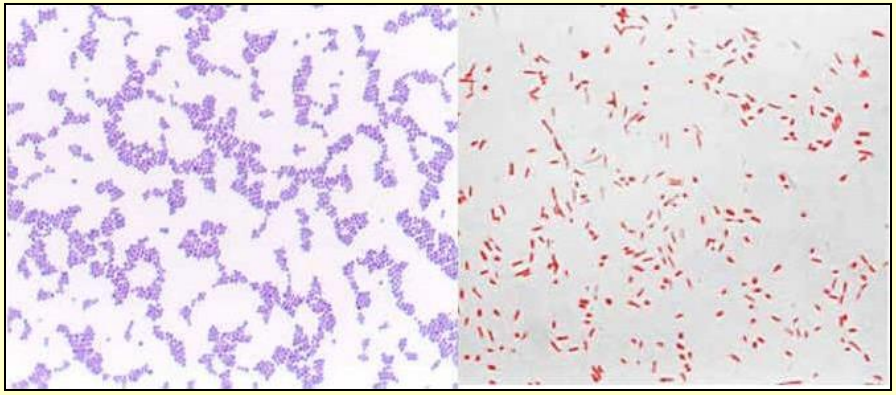
IMMUNE RESPONSE (either an aggressive response or immunological tolerance)

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- **Gram-**



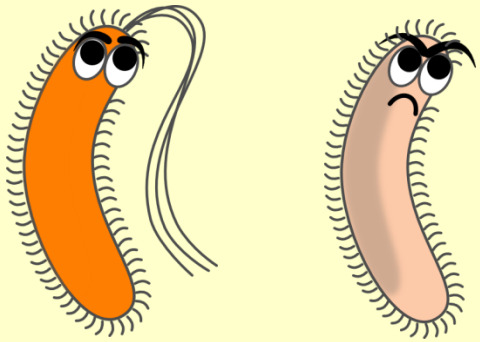
The **Gram staining** is used to differentiate bacteria based on the **chemical properties of their cell walls**.

E.g.:

Staphylococcus aureus, Streptococcus pneumoniae

Escherichia coli, Salmonella enterica

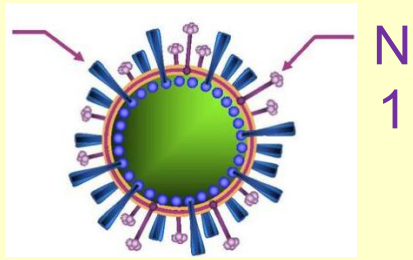
Not all bacteria cause diseases in healthy individuals with a well-functioning immune system, but almost all bacteria can be pathogenic in immunocompromised patients.



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.])

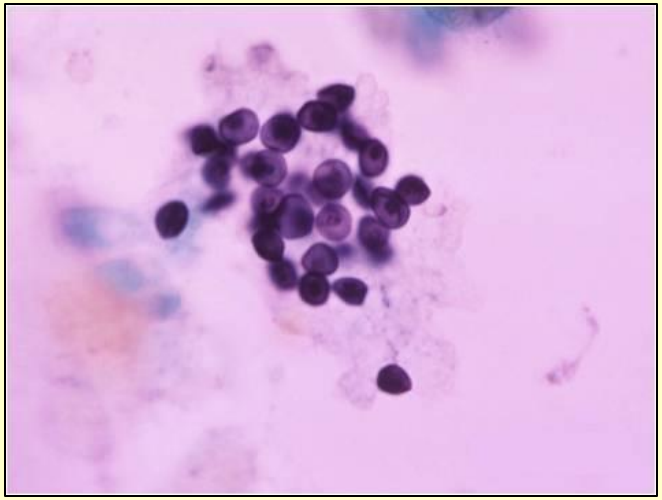
What threatens us? II.

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

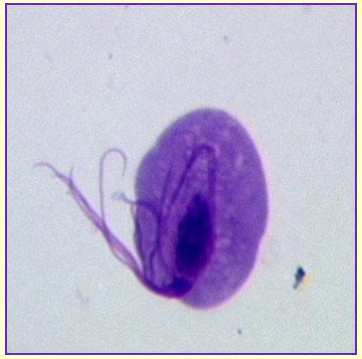
3. **Fungi**
   - Roughly 1,5 million species of fungi live on Earth with approx. 300 being pathogenic to humans.
   - Severe fungal infections mostly occur in **immunodeficient patients**.[3.]



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.]



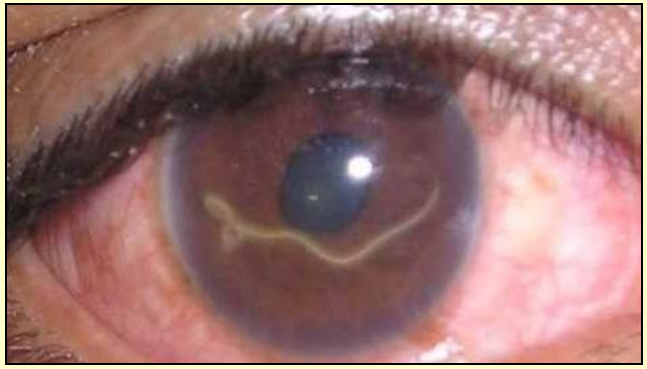
The flagellated *Trichomonas vaginalis,* causative agent of Trichomoniasis which is the most common non-viral STD with 248 million cases each year worldwide.[9.]

5. **Multicellular parasites**

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

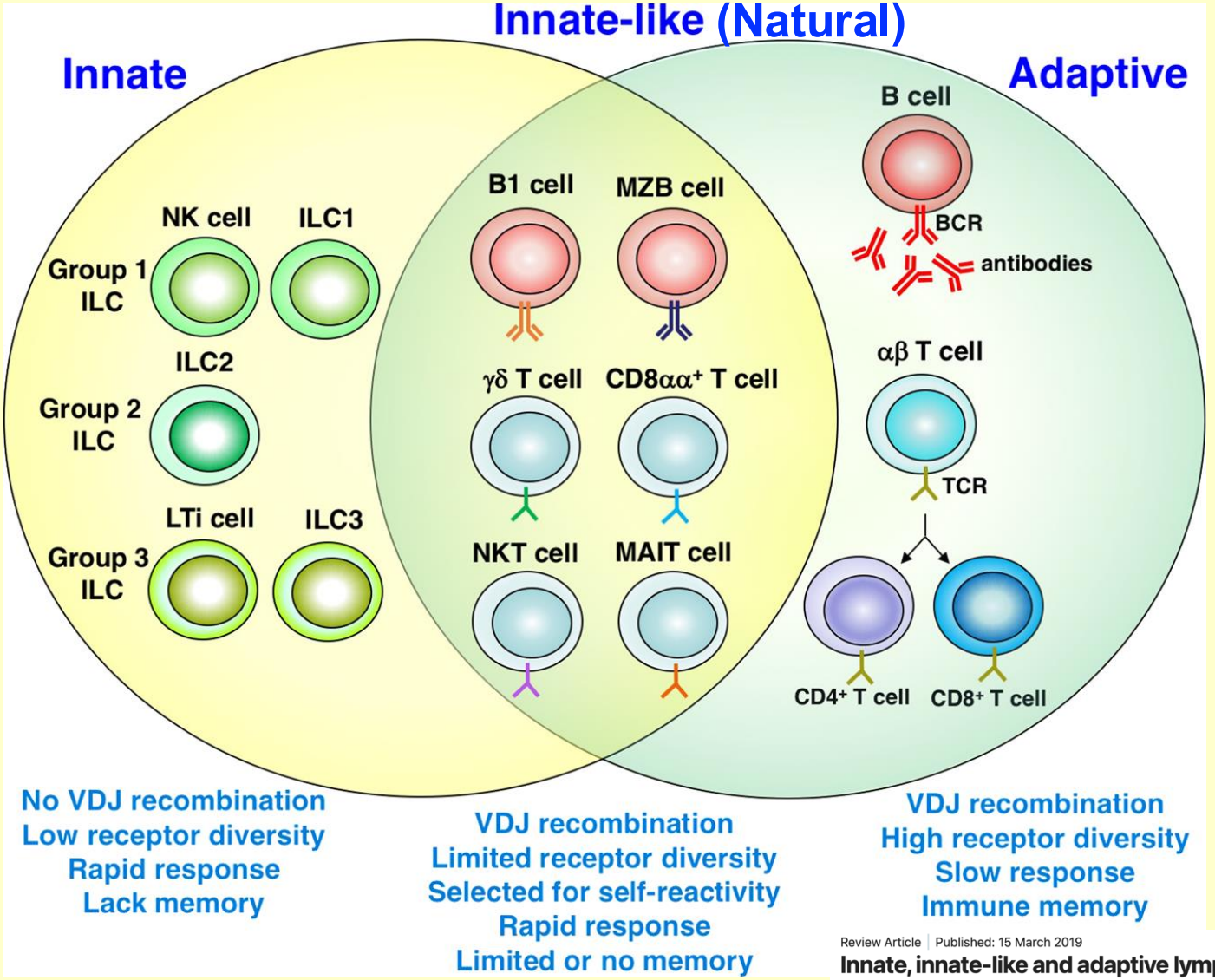
6. **Prion**

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.])

Lymphocyte groups



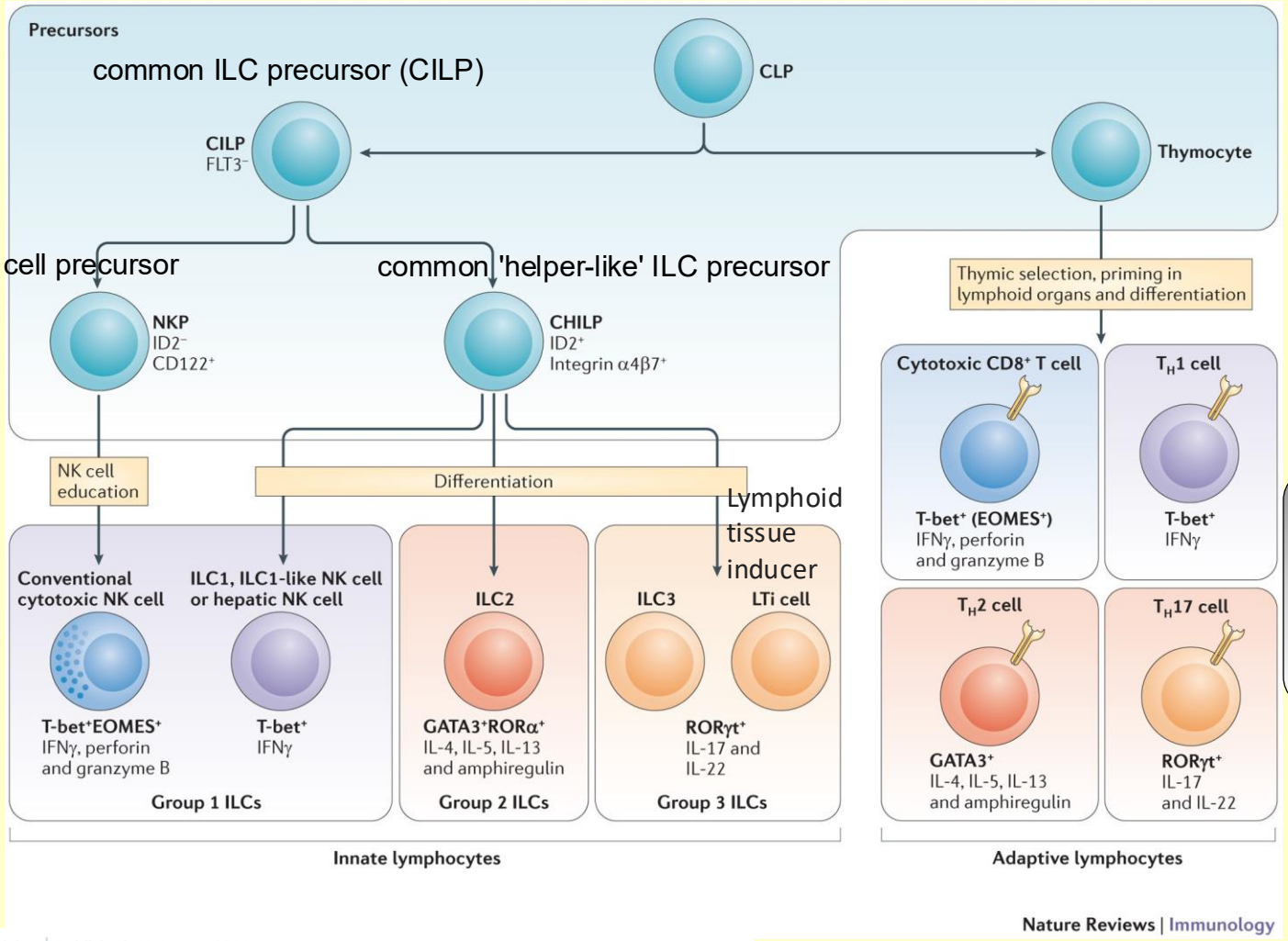
Review Article | Published: 15 March 2019

Innate, innate-like and adaptive lymphocytes in the pathogenesis of MS and EAE

Luc Van Kaer ✉, Joshua L. Postoak, Chuan Wang, Guan Yang & Lan Wu

Cellular & Molecular Immunology **16**, 531–539 (2019) | Cite this article

Differentiation of lymphocytes



Treg

FoxP3+
IL-10, TGFβ

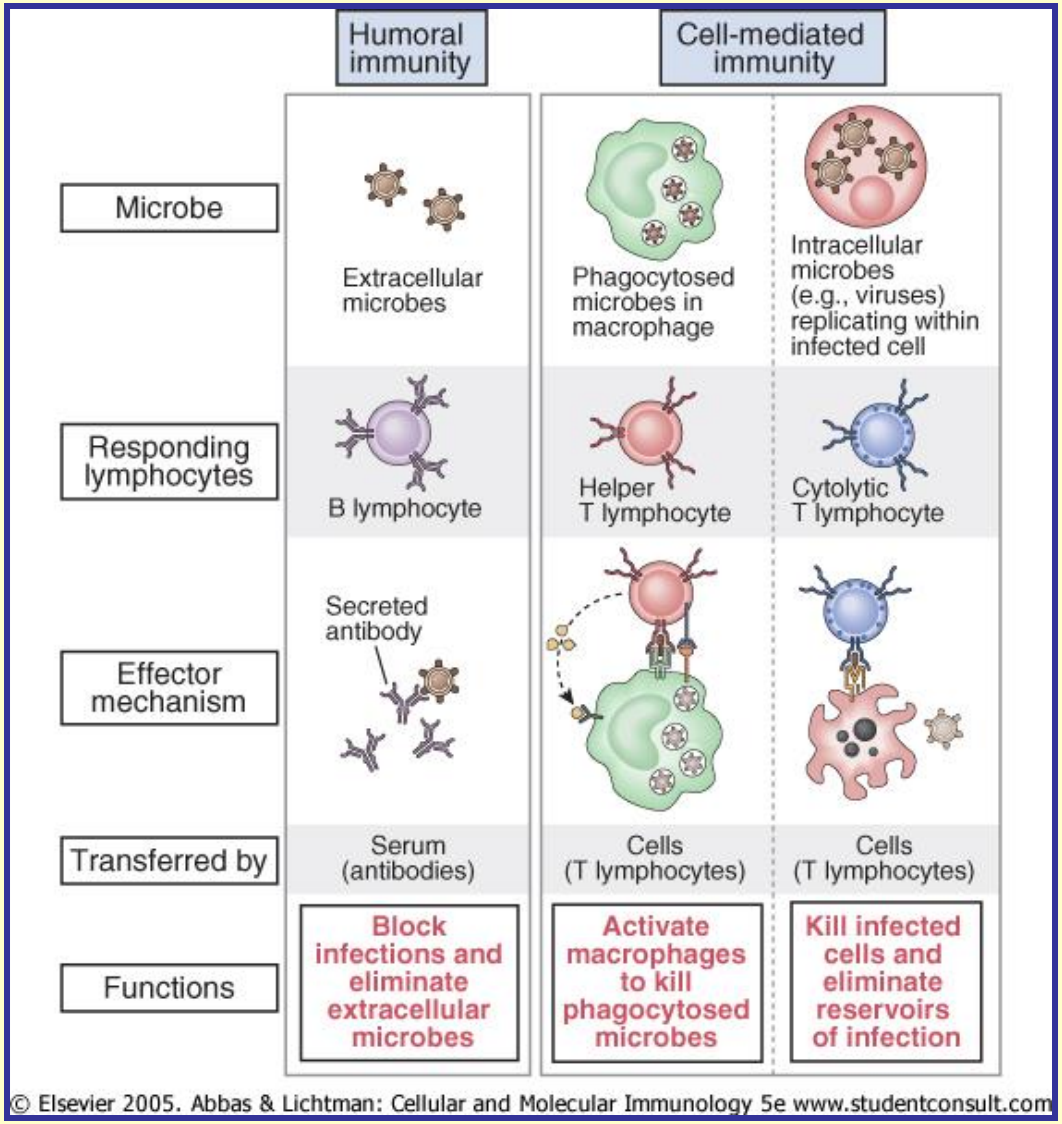
Opinion | Published: 18 August 2014

Interactions between innate and adaptive lymphocytes

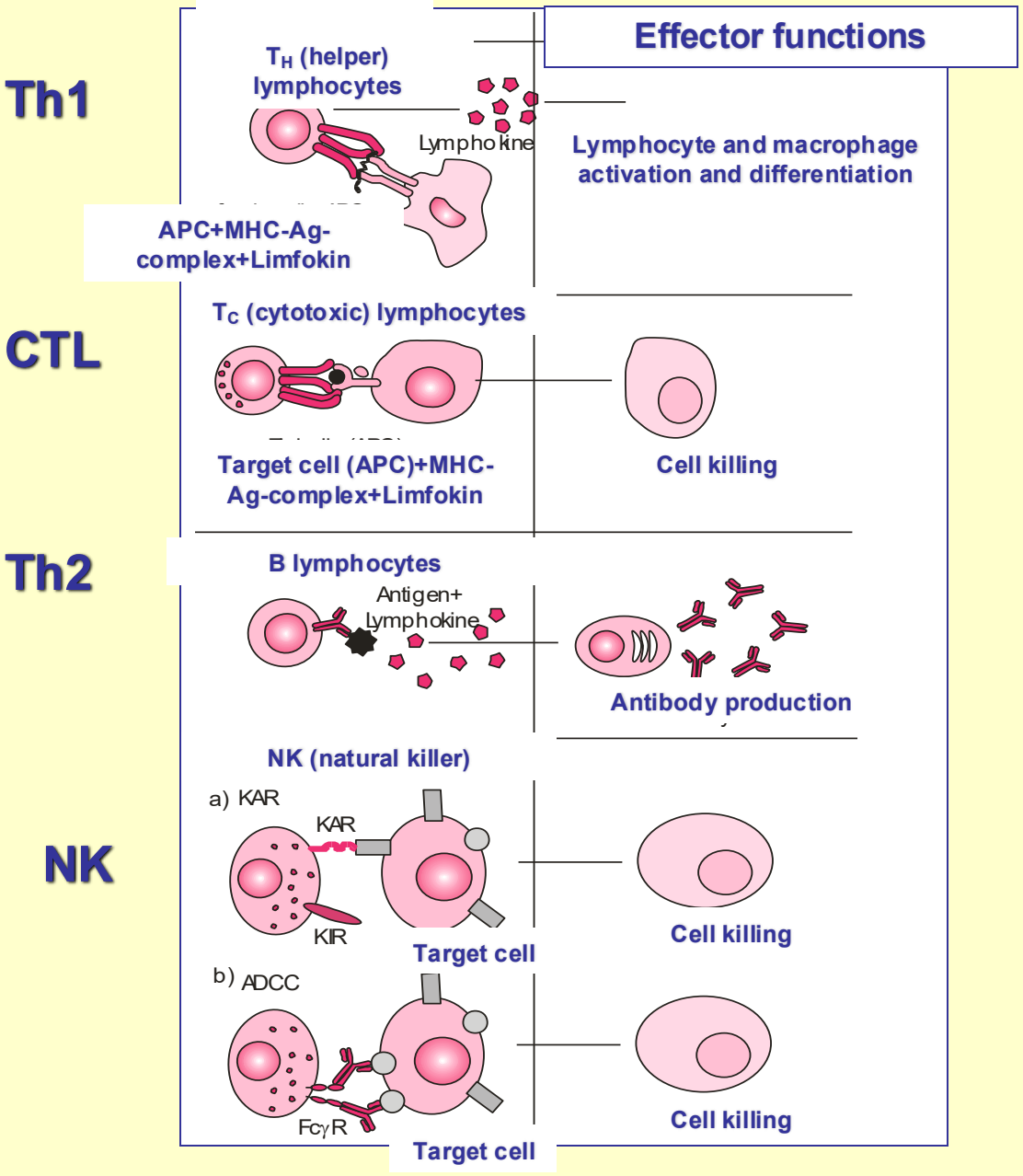
Georg Gasteiger & Alexander Y. Rudensky

Nature Reviews Immunology **14**, 631–639 (2014) | Cite this article

The type of pathogens determine the type of immune response



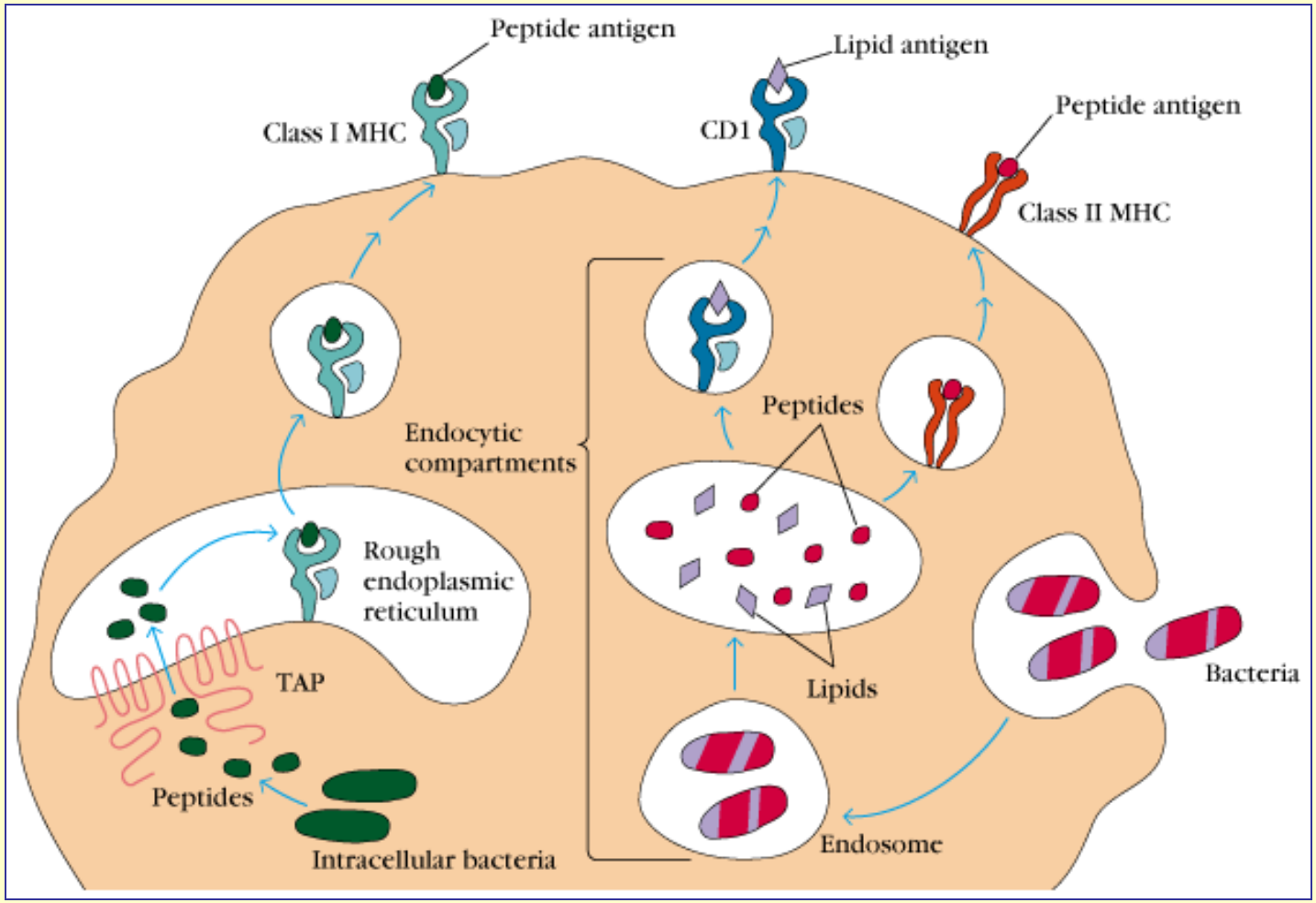
Effector functions of lymphocyte populations



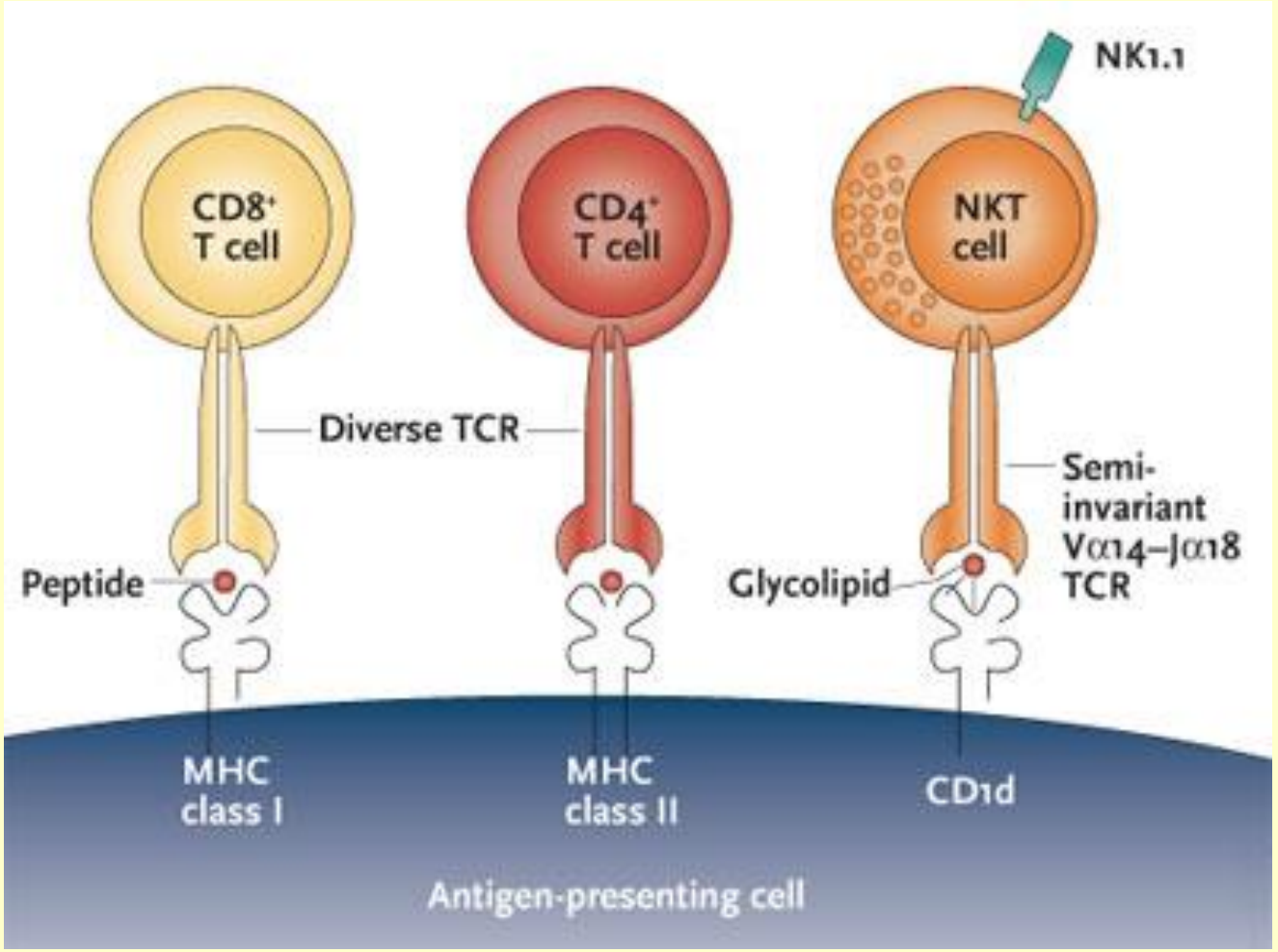
Cell-mediated immuneresponse (CMI)

Cytotoxicity	***DTH***
Effector cells direct cytotoxic activity: - CTL (CD8+ Tc), - γδ T cells - NK cells, - Macrphages	**Effector cells** cytokine production: - T_{DTH} cells = Th1 cells - Macrophages
Target cell (cytosolic antigen): - allogen cells (transplantation minor histocompatibility antigen) - malignant cells - virally infected cells - chemically modified cells	**Antigen in phagolysosome:** - intracellular bacterium, fungi, parasite, virus - contact antigens (small molecules (haptén) skin protein complexes)

Presentation of intracellular and extracellular antigens



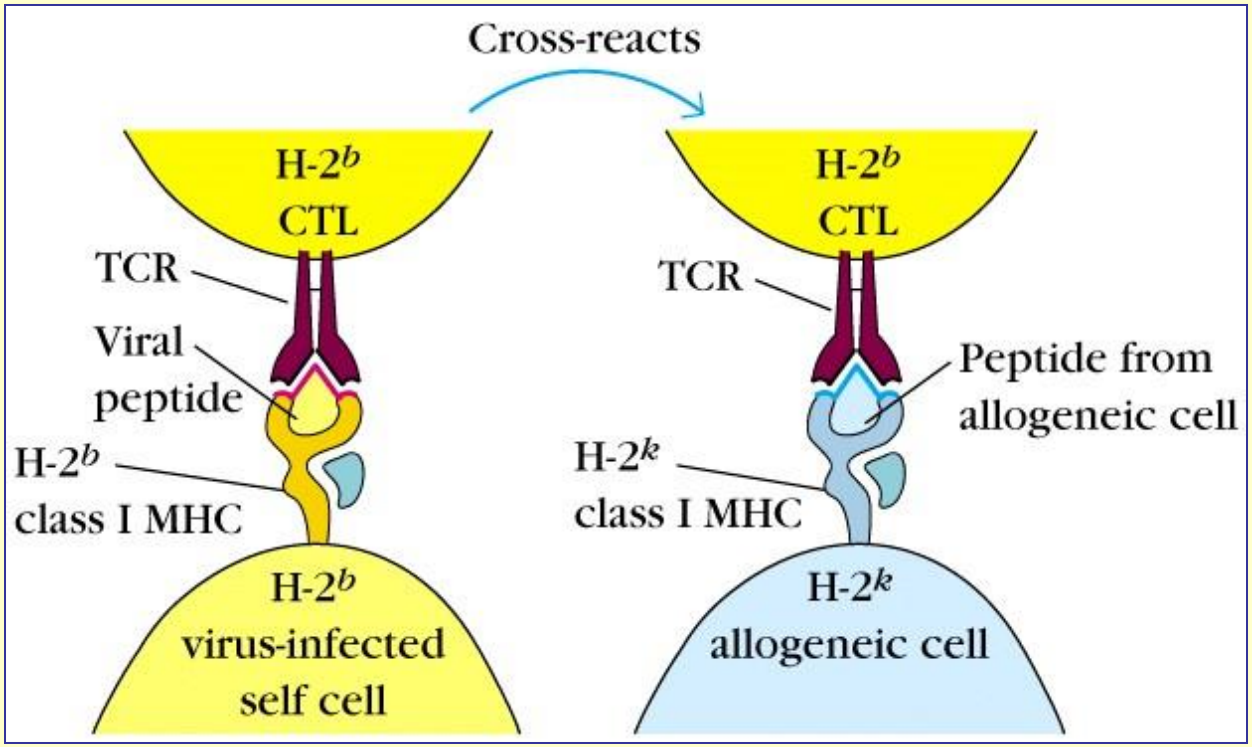
Cytosolic way **Phagolysosomes**


NK1.1
CD8+ T cell
CD4+ T cell
NKT cell
Diverse TCR
Semi-invariant Vα14–Jα18 TCR
Peptide
Glycolipid
MHC class I
MHC class II
CD1d
Antigen-presenting cell

Cytotoxicity

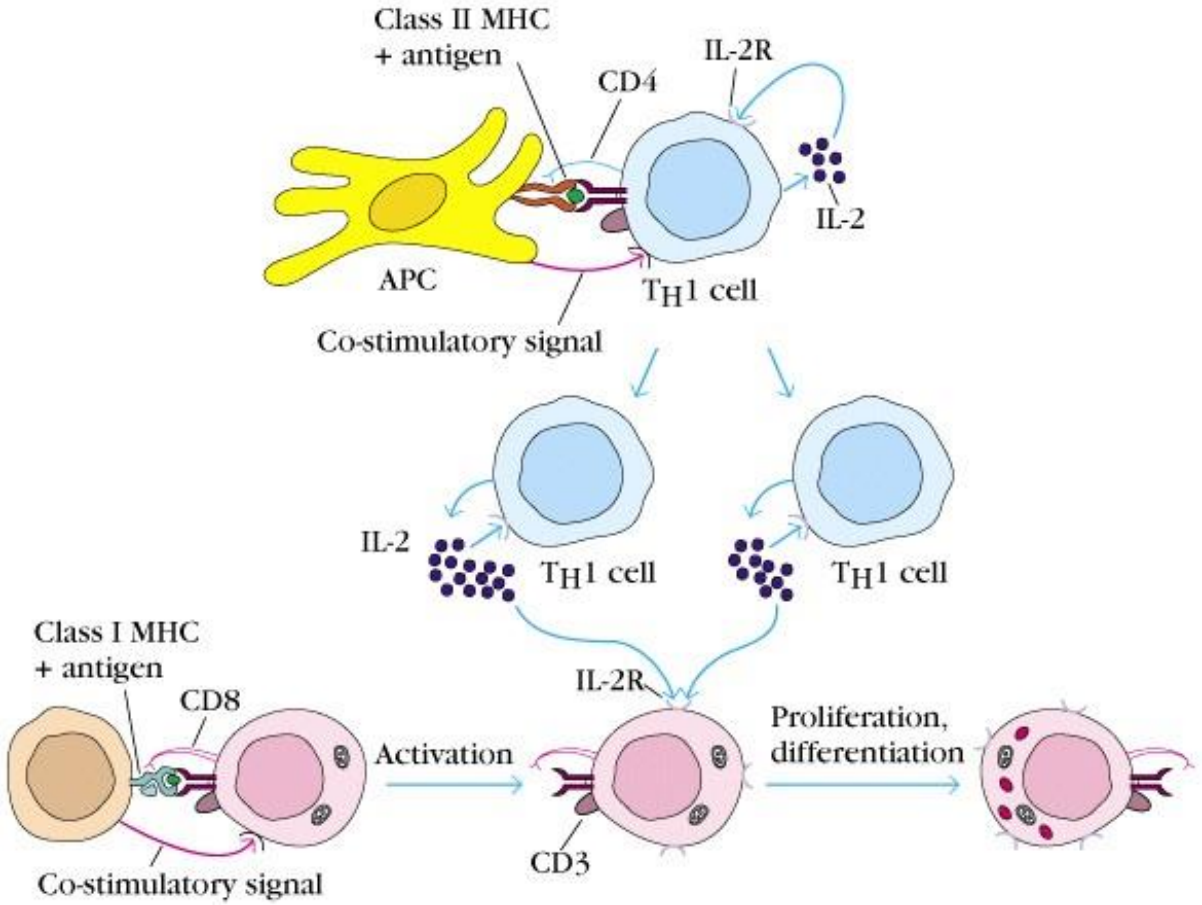
CD8+ T cells

Antigen recognition of cytotoxic T cells



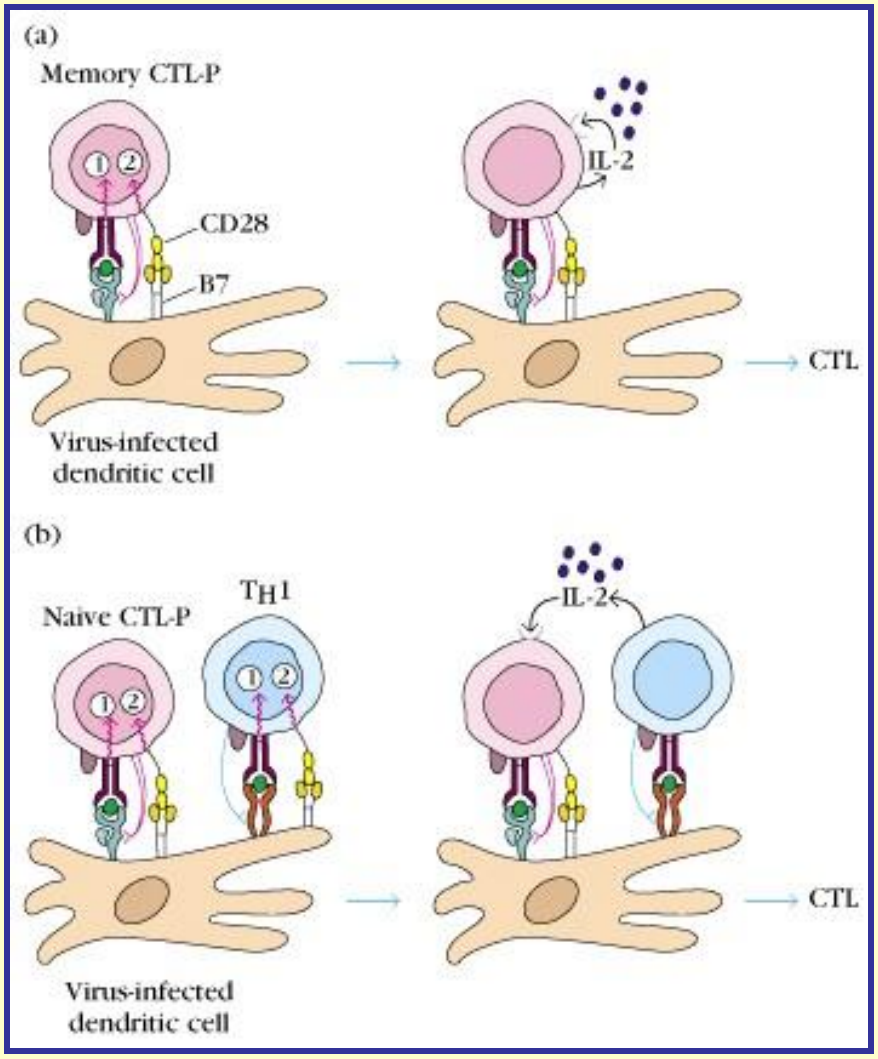
Activated Tc cells = effector CTL
TcRαβ, CD8+ cells
Antigen specific recognition with MHC- I restriction

Naive Tc cell → effector CTL



IL-2R expression	–	+	+
IL-2 expression	–	±	±
Proliferation	–	–	+
Effector cytotoxic function	–	–	+

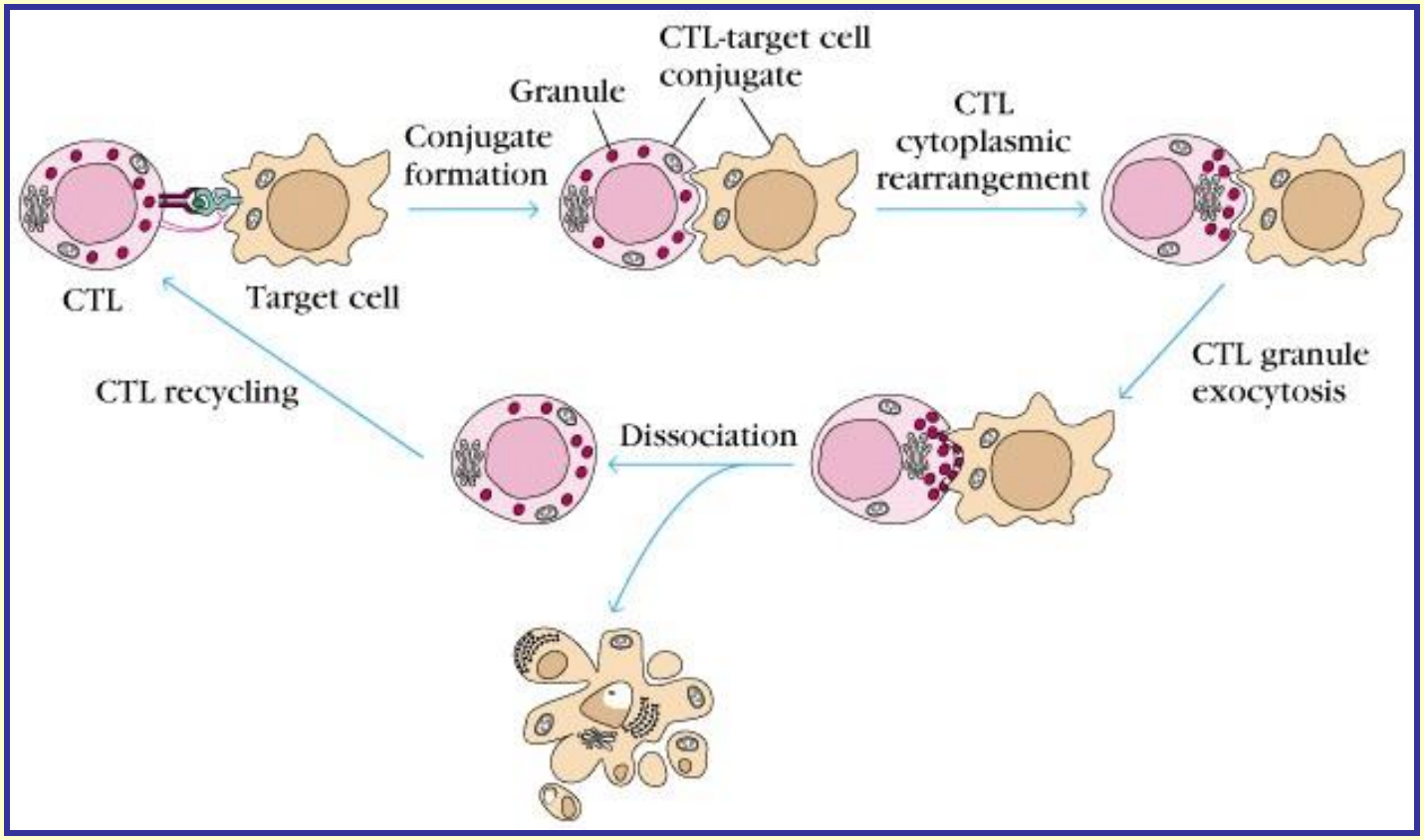
Activation of memory CTL doesn't require Th1 help



Memory CTL: autokrin IL-2 production

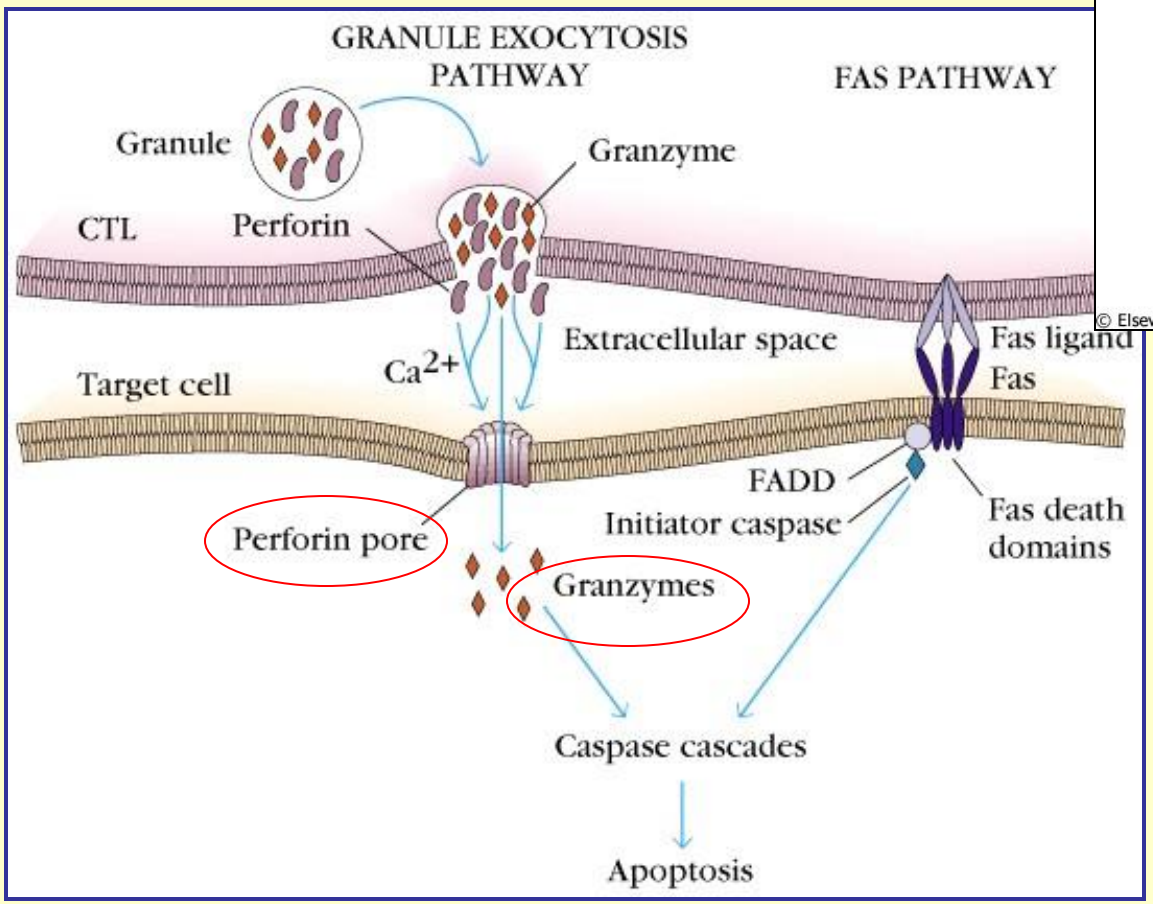
Naiv CTL: Th1 produces IL-2

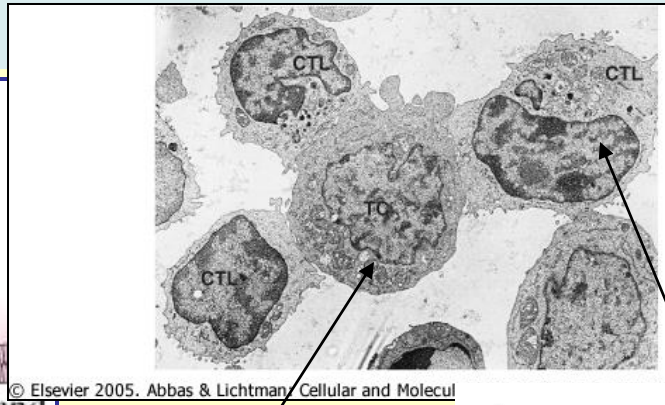
CTL-mediated target cell killing:



1. Antigen recognition 2. Conjugation 3. CTL cytoplasmic rearrangement
4. CTL degranulation 5. Target cell apoptózis 6. Dissociation

Mechanisms of CTL induced apoptosis:





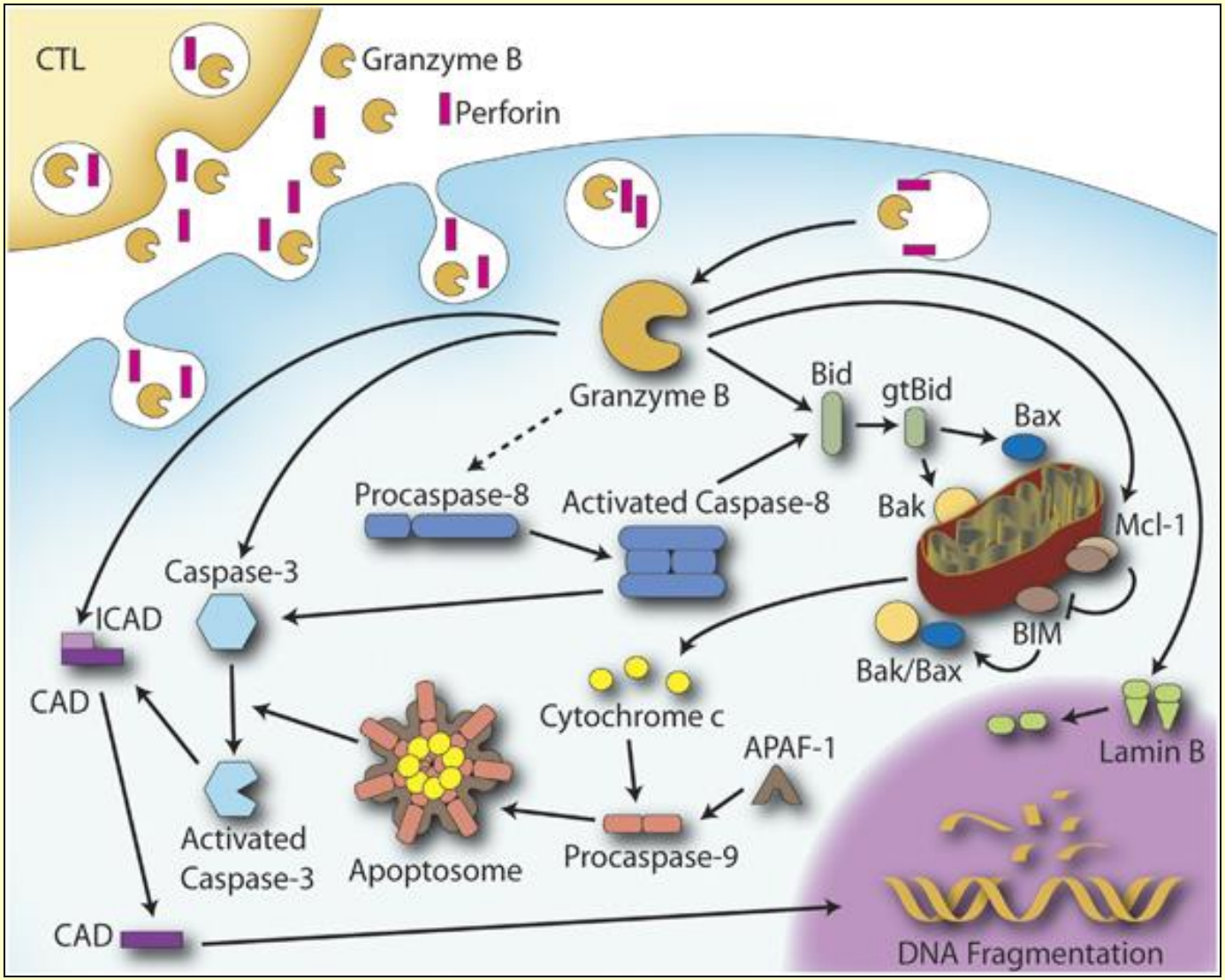
Target cell

Cytotoxic T-cell

Soluble effector molecules: perforins and granzymes

Membrane-bound effector molecules: Fas/Fas ligand (FAS-L)

The secretory mechanism of apoptosis

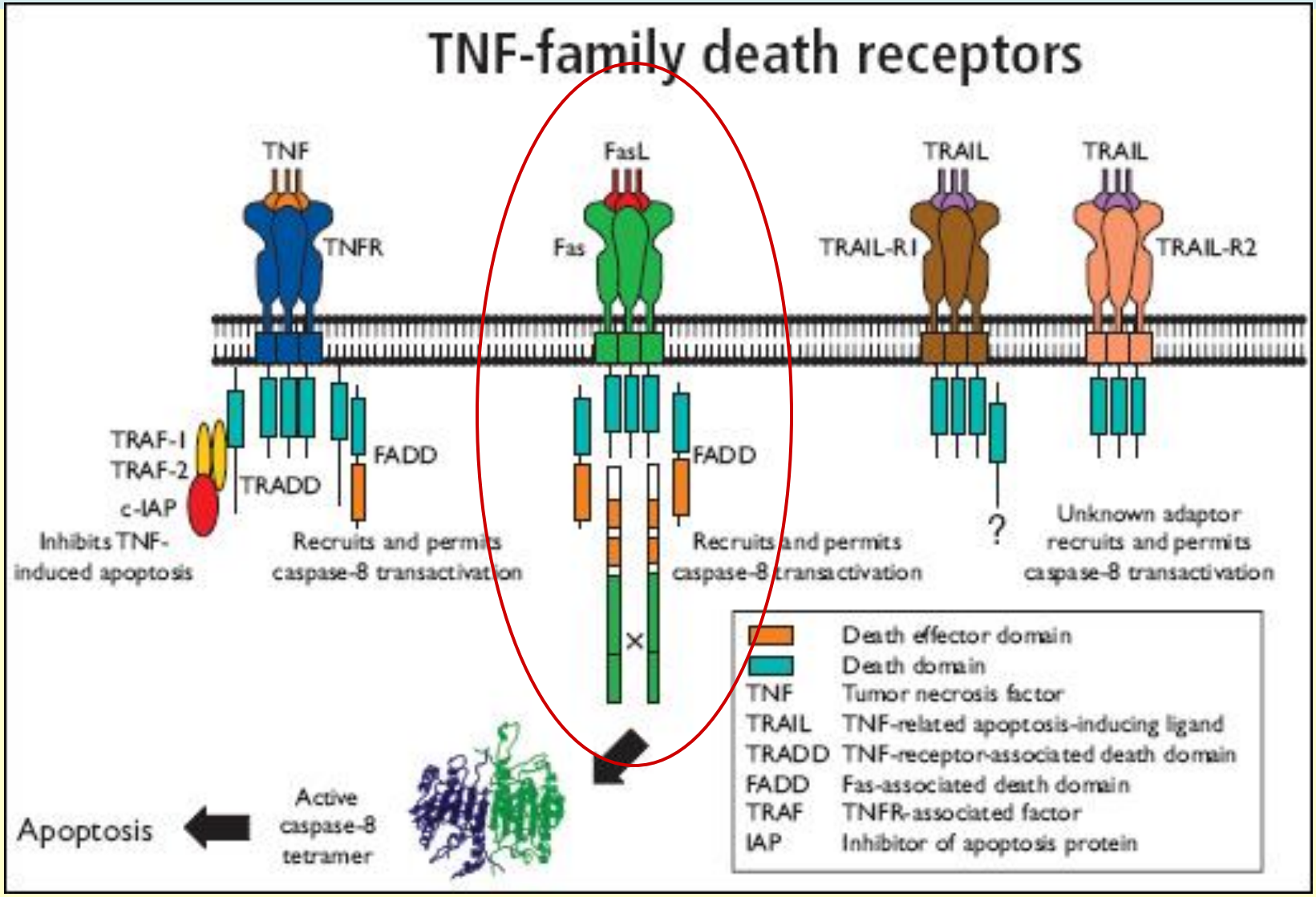


Granzyme B:

Induction of Apoptosis

Granzyme A:
DNA-Fragmentation

Extrinsic Apoptosis pathway



Caspase Activated Deoxyribonuclease (CAD)

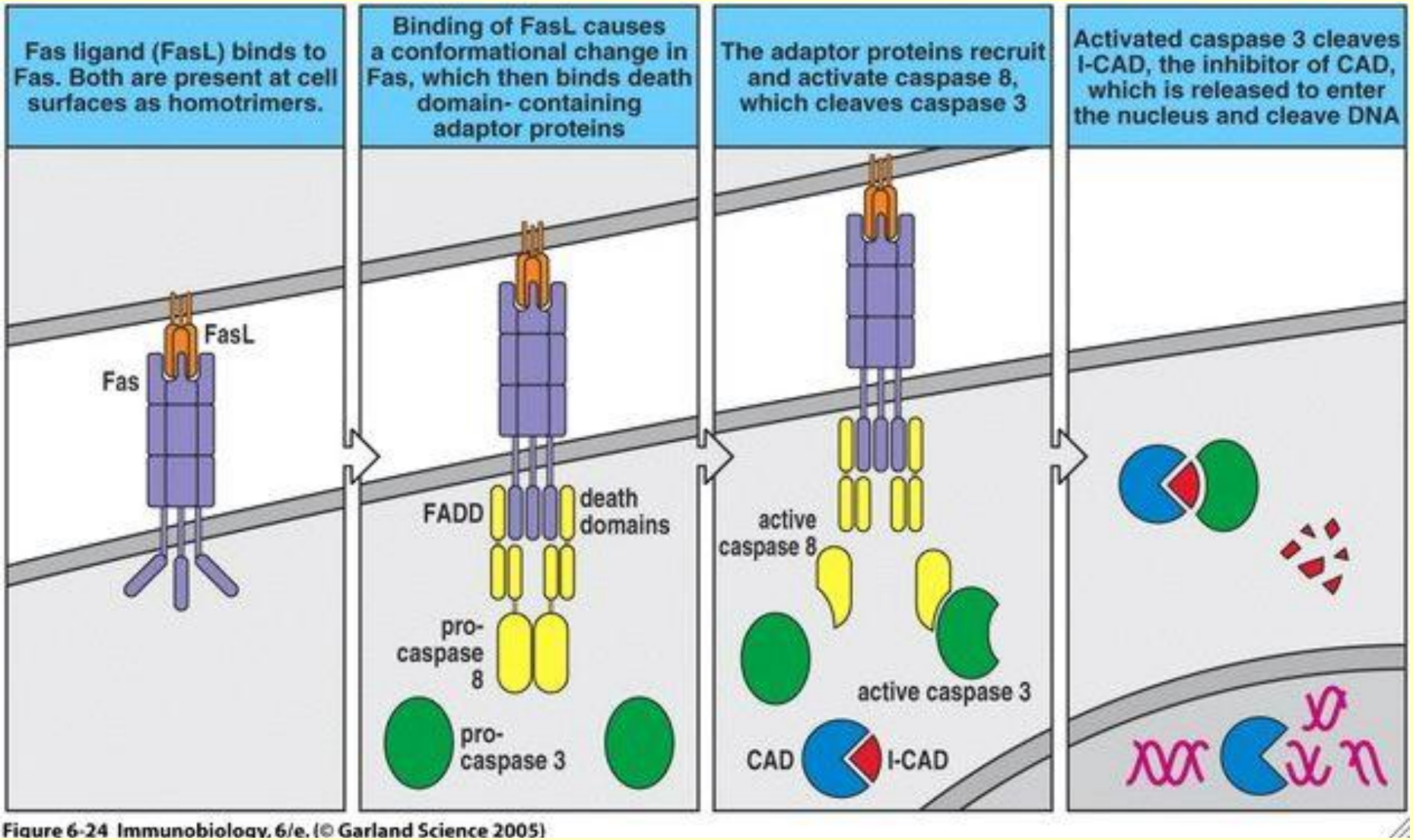


Figure 6-24 Immunobiology, 6/e. (© Garland Science 2005)

When activated by caspase-3, CAD is responsible for cleaving DNA into the characteristic ~200 bp fragments of apoptotic cells.

Cytotoxicity

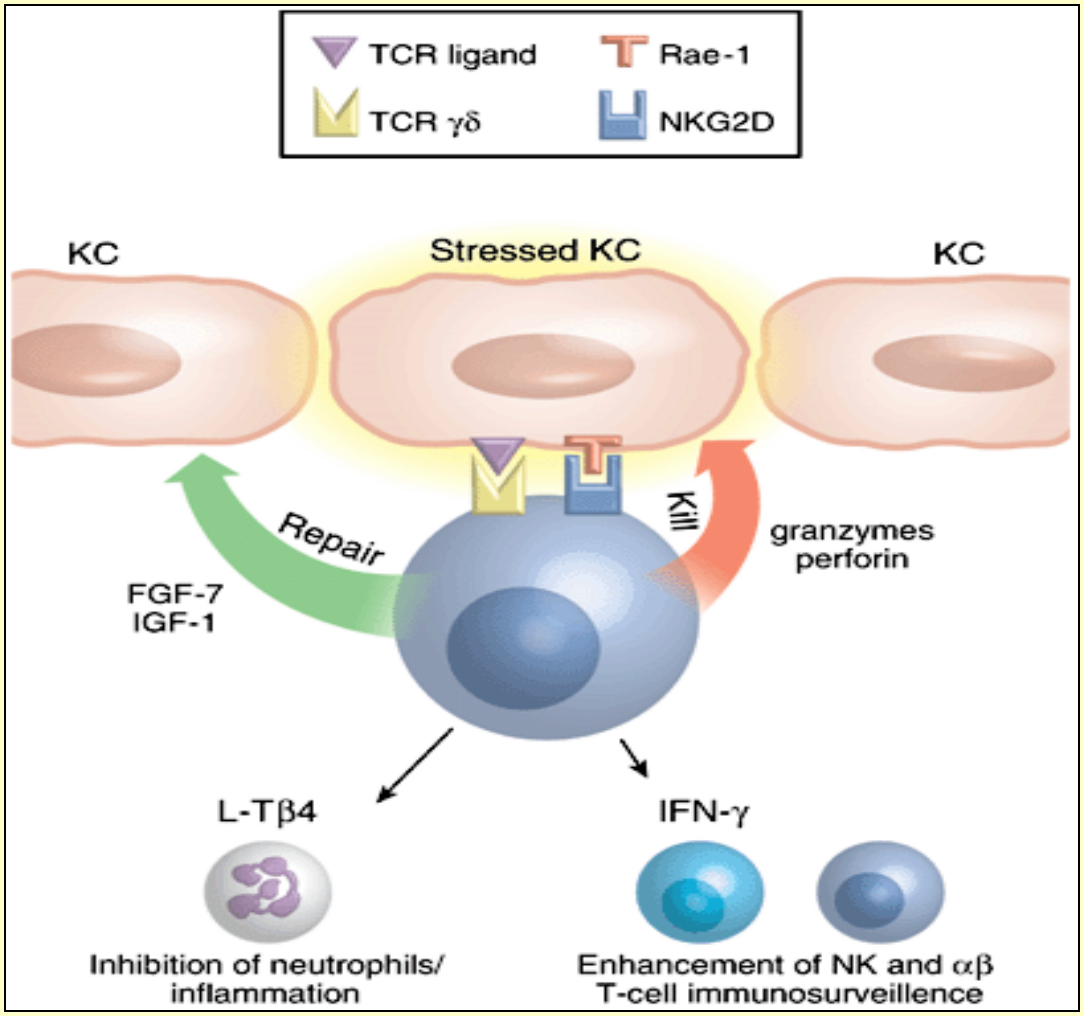
$\gamma\delta$ T cells

γδ T cells

- 5 % of the T cells,
- Intraepidermal lymphocytes: CD4- and CD8-
- Intraepithelial lyphocytes: CD8+
- Produced in embryonic life, no recirculation,
- Limited, tissue specific TcR diversity → specialization to respond to certain antigens

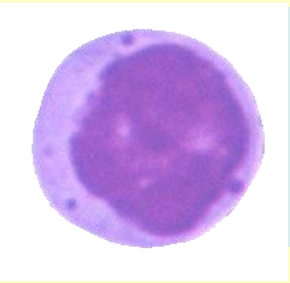
- Ligand recognition: - non-MHC-retricted, but antigen specific
- Antigens: viral proteins, surface heat-shock proteins (produced in inflammatory responses) bacterial lipids, phosphatids through CD1 molecule
- Function: eliminate damaged cells and microbial invaders

γδ T cells

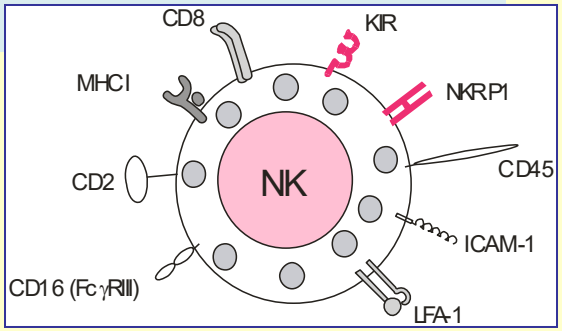

TCR ligand
Rae-1
TCR γδ
NKG2D
KC
Stressed KC
KC
Repair
FGF-7
IGF-1
Kill
granzymes
perforin
L-Tβ4
IFN-γ
Inhibition of neutrophils/
inflammation
Enhancement of NK and αβ
T-cell immunosurveillence

Cytotoxicity

NK cells



Natural killer cells (NK)



- 10-15% of lymphocytes = LGL cells

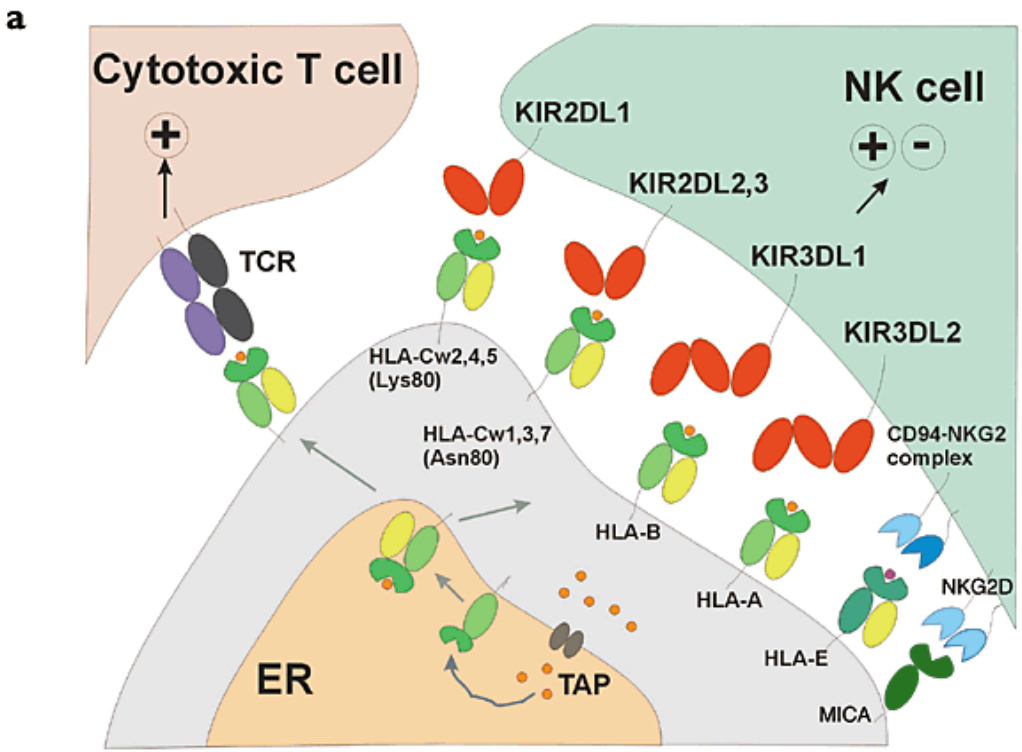
- **Phenotype:**
- TcR- CD3-, CD4-, CD8+/-, CD2+, CD16+ (FcγRIII) CD56+,
- They secrete cytokines: INFγ → immune regulation (Th1)

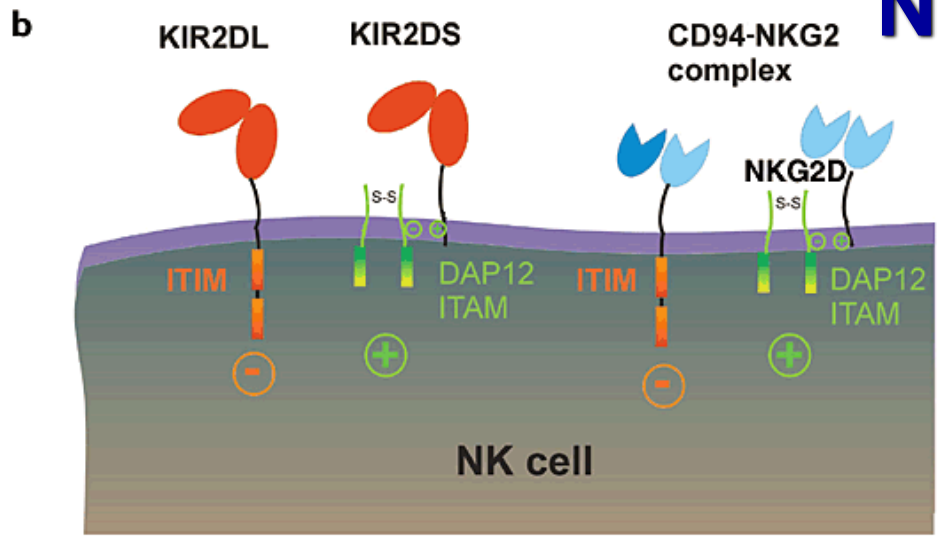
- **Function:** ***early*** response to infection with certain viruses, intracellular bacteria and tumor cells

NK-cell receptors:

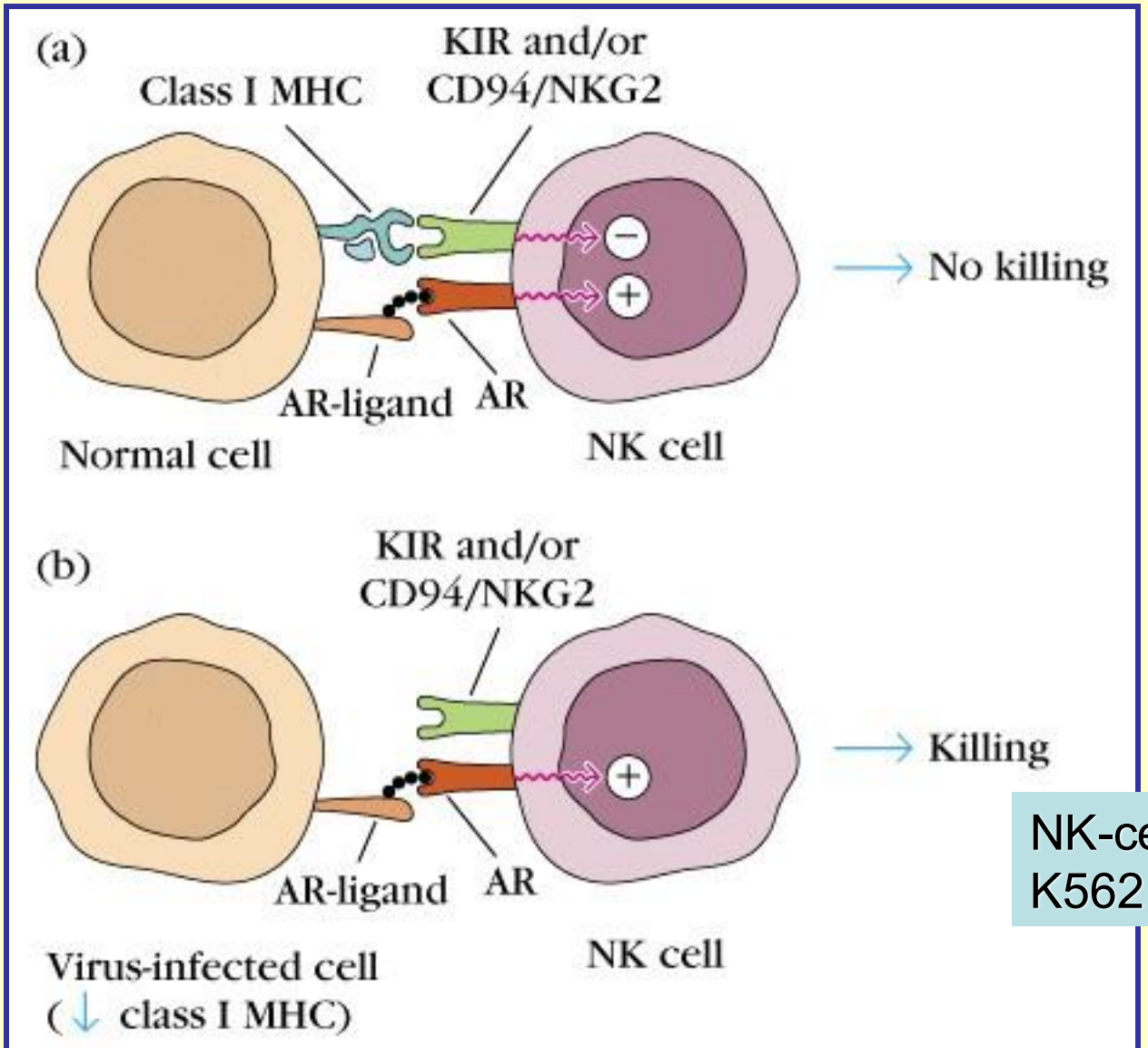
- **Killer inhibitory receptors (KIR):** recognize normal self MHC-I molecules
- **Killer activatory receptors (KAR):** recognize aberrant glycosylation on tumor or virus infected cell surface

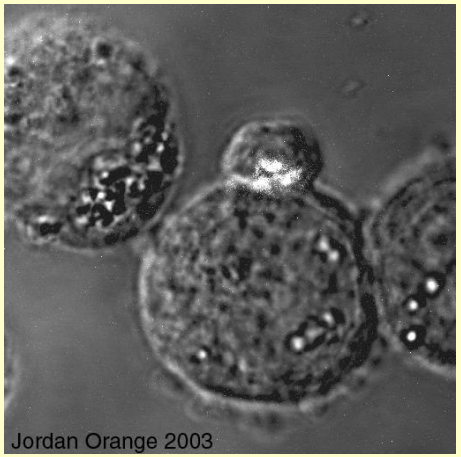
NK cell receptors:





KIR: killer inhibitory receptors and their ligand





NK-cells kill their target-cell K562 with perforin (white)

Antibody-dependent cellular cytotoxicity (ADCC)

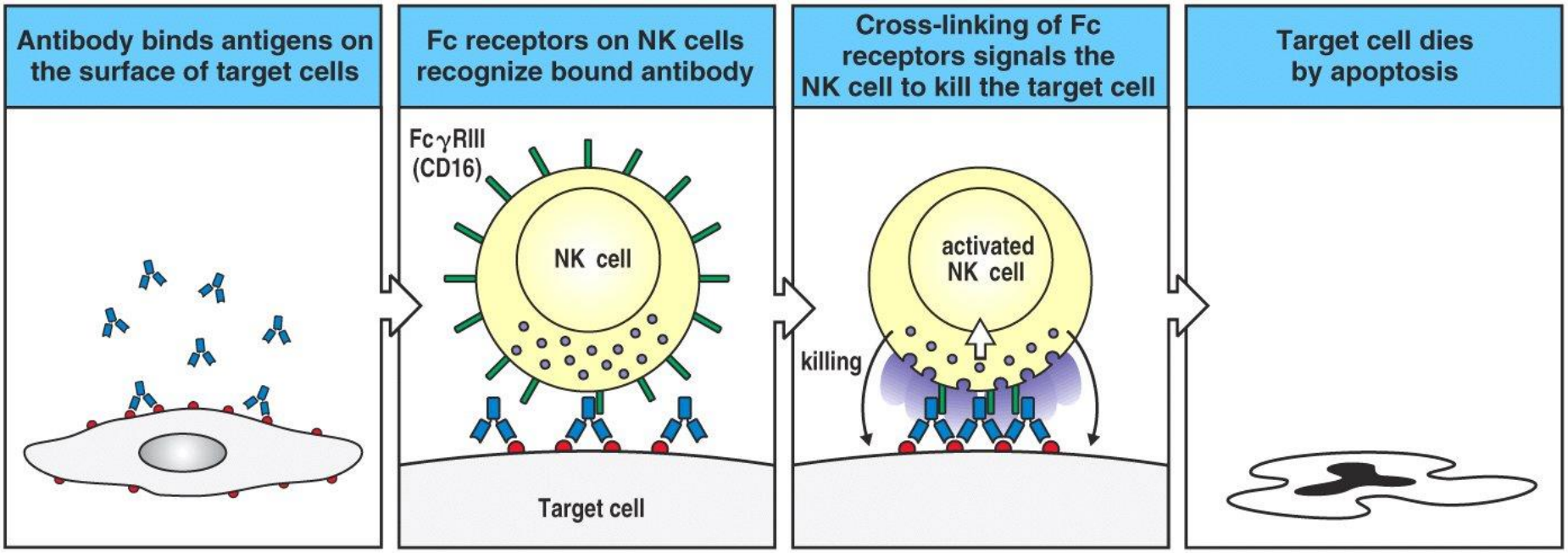
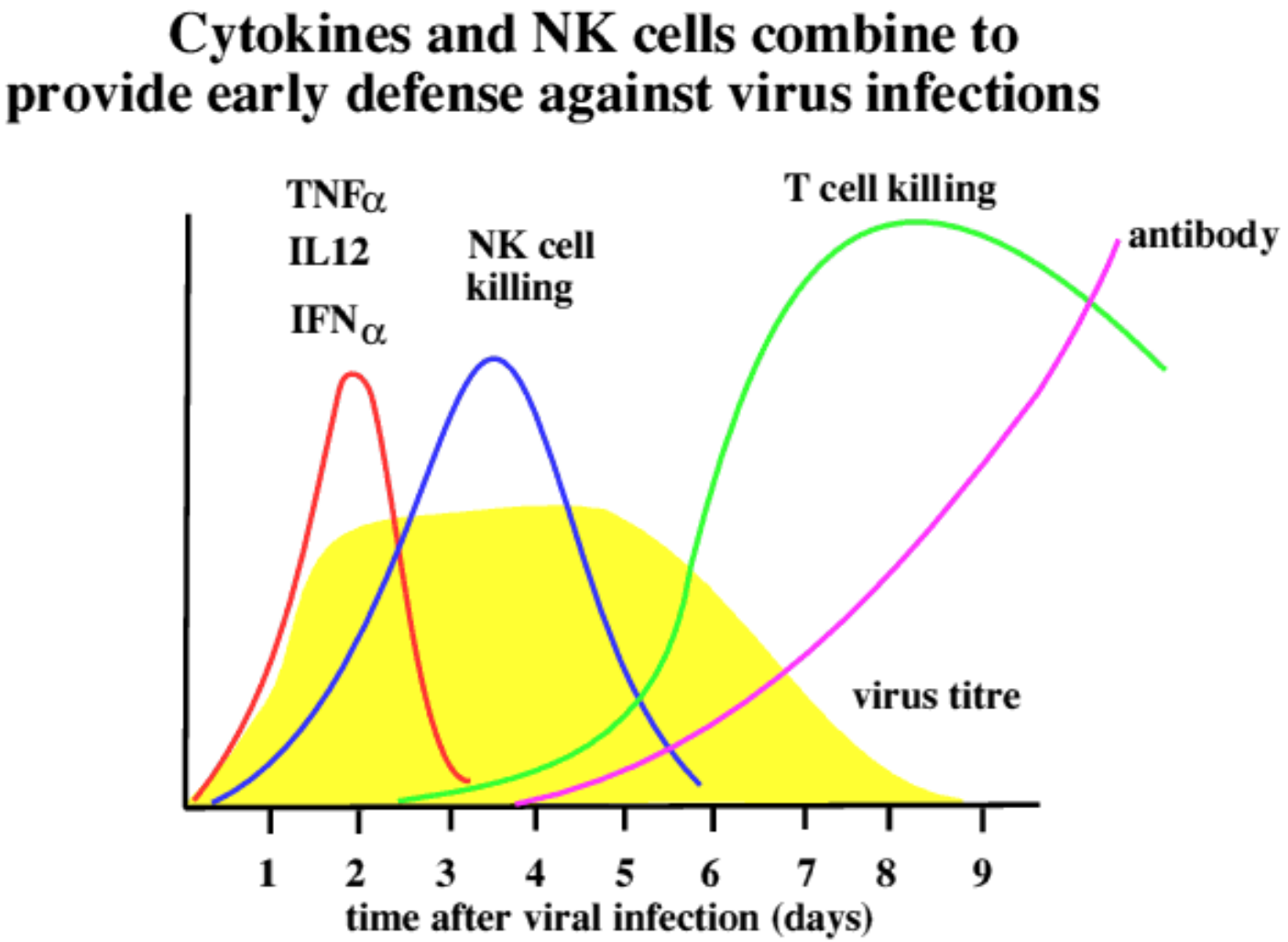
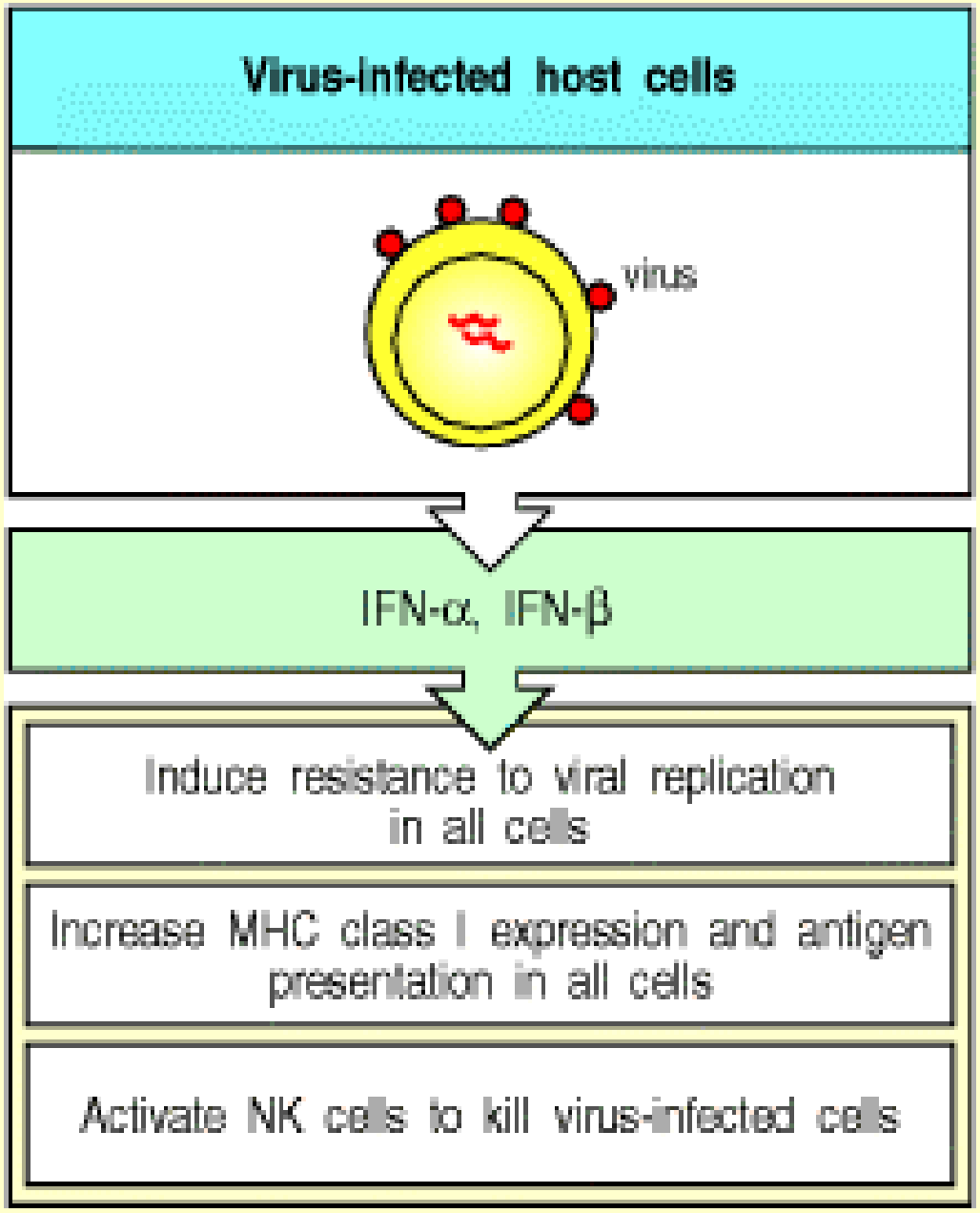


Figure 9-34 Immunobiology, 6/e. (© Garland Science 2005)

The time-kinetic of the immune response against viruses




Virus-infected host cells
virus
IFN-α, IFN-β
Induce resistance to viral replication in all cells
Increase MHC class I expression and antigen presentation in all cells
Activate NK cells to kill virus-infected cells

Cytotoxicity

NKT cells

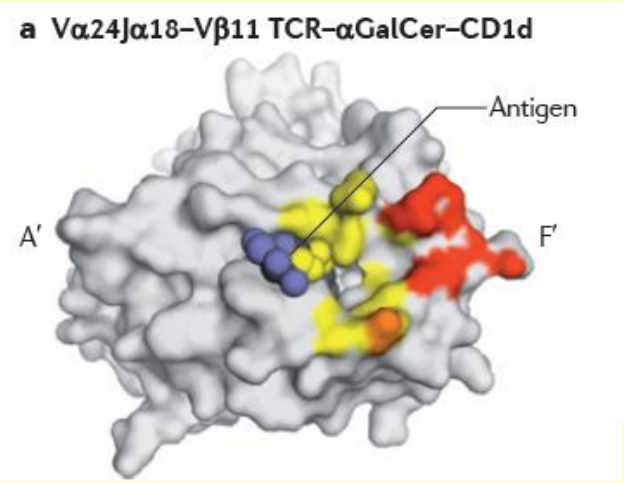
Natural Killer T cells = NKT

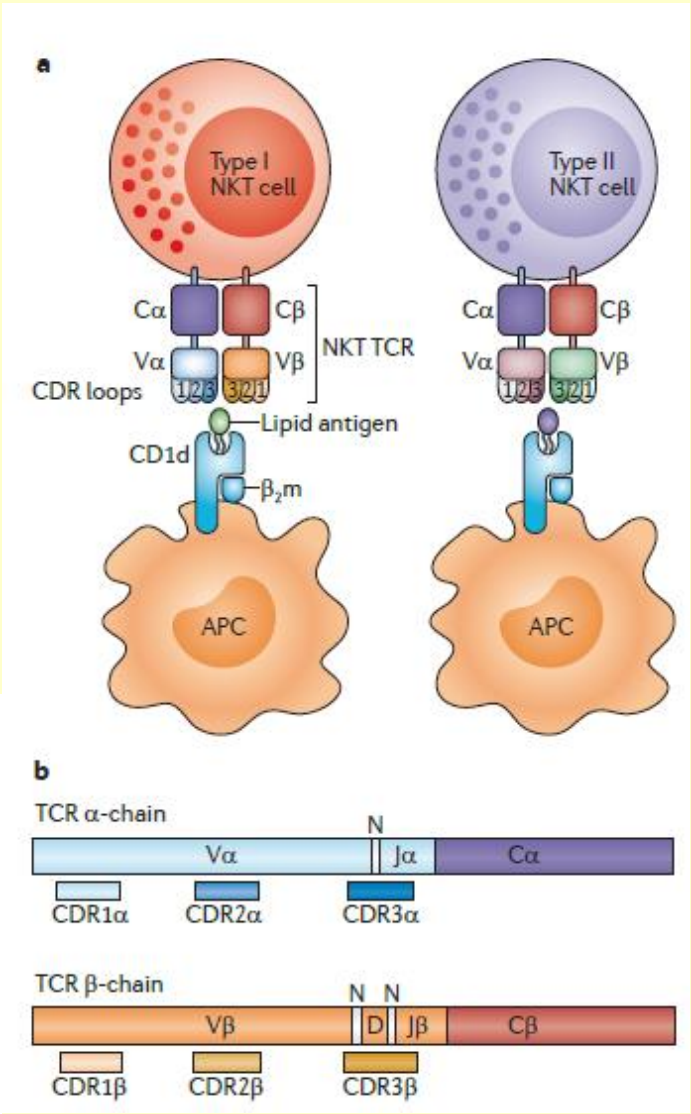
- 0,2% of the peripheral T cells
- Positive selection in the thymus on self phospholipid antigens
- **Antigen recognition**: microbial **phospholipids** and **glycolipids**, presented by the non-polymorphic **CD1d**
- **Markers:** invariant αβ TcR (iVα24-Jα18) with limited specificity, CD4 or DN or CD8αα + NK markers: NK1.1, CD56, CD16, CD161 (NKRP1)
- **Function**: fast cytokine production: IL-4, IFNγ, IL-10, IL-13, IL-17, IL- 21 TNFα

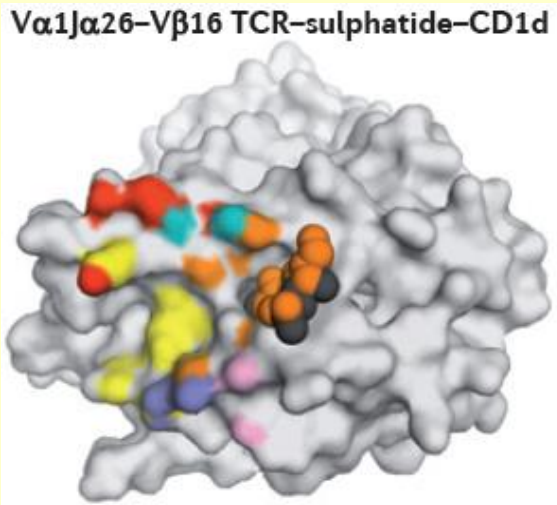
	Vα14 NKT	Conventional T
TCR	invariant Vα14	heterogenous TCR
Ligand	α-GalCer	peptides
MHC	monomorphic CD1d	polymorphic MHC
Major tissues	Liver, Spleen Bone marrow	Thymus, Spleen Lymph nodes
Development	GM-CSFR	no GM-CSFR

Natural Killer T cells = NKT

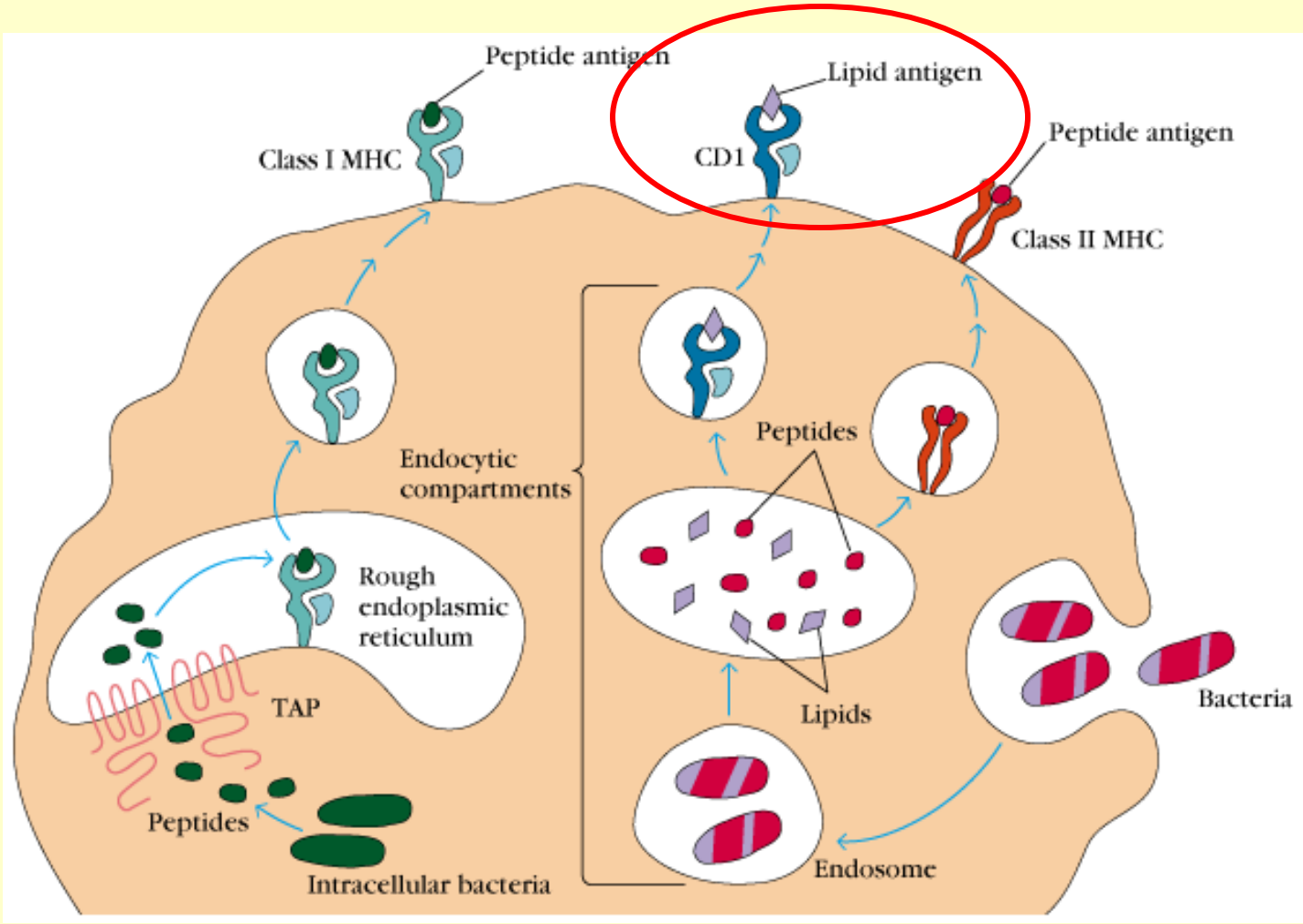
(iVα24-Jα18) had been reported in human DN T cells

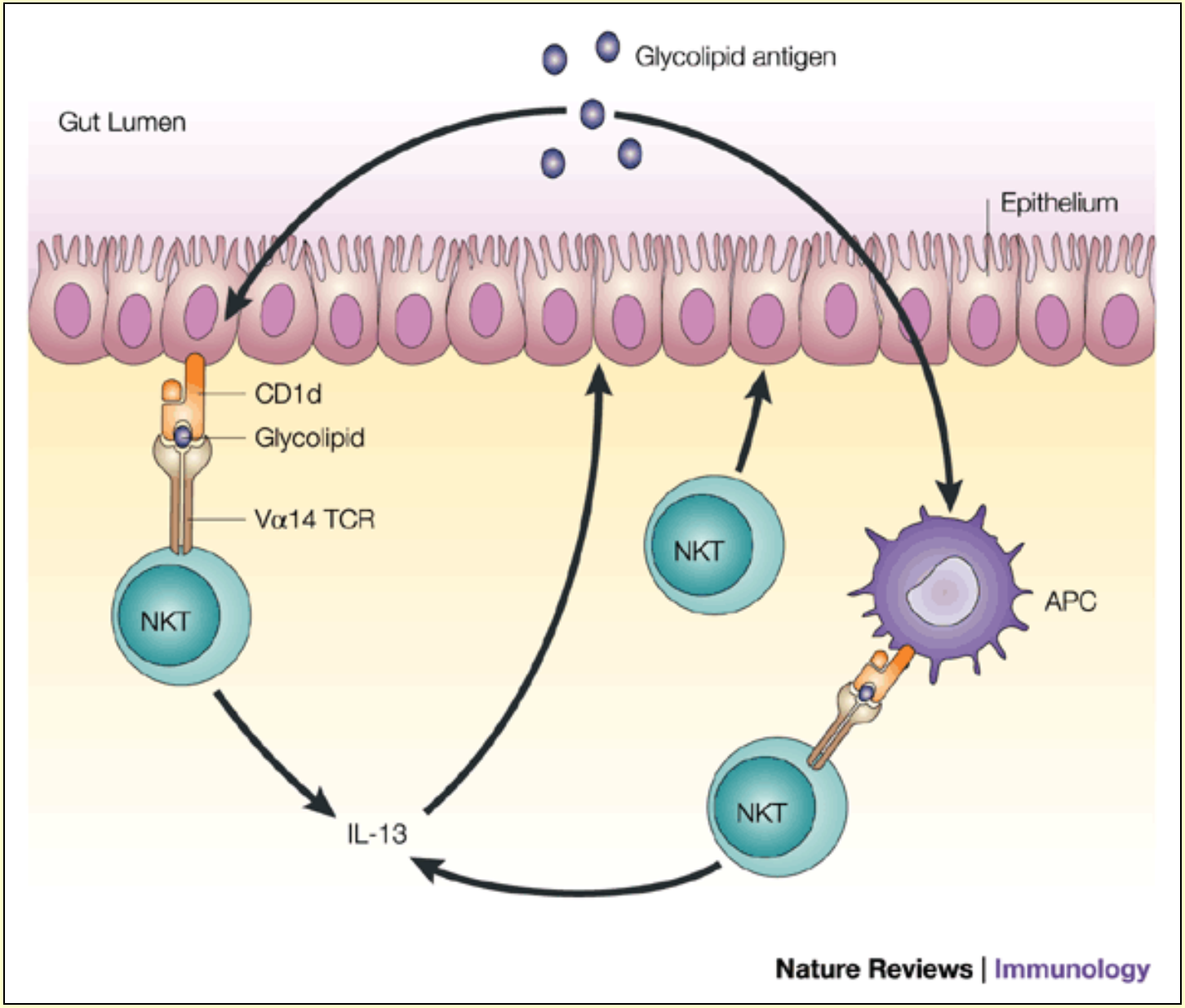






Bacterial lipid antigen presentation by CD1




Glycolipid antigen
Gut Lumen
Epithelium
CD1d
Glycolipid
Vα14 TCR
NKT
NKT
APC
NKT
IL-13
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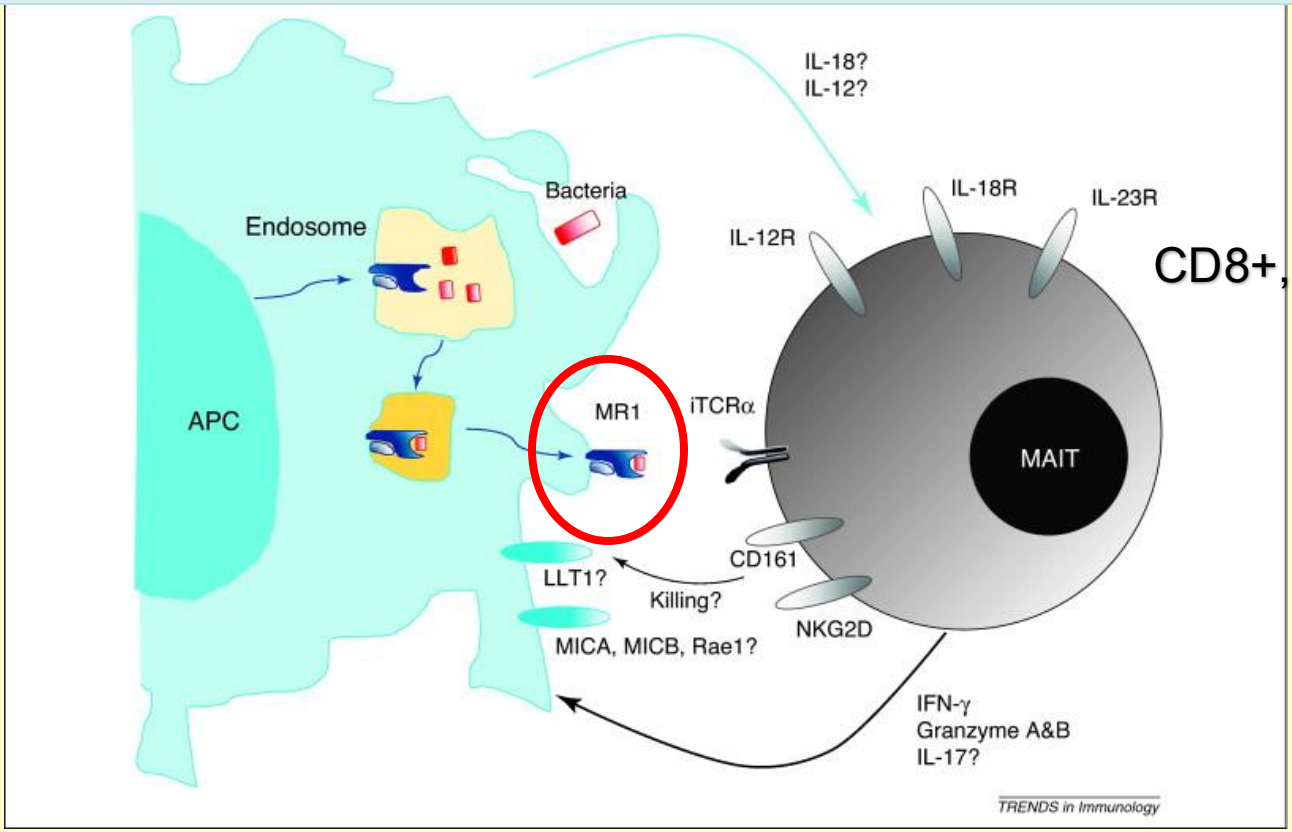
Cytotoxicity

MAIT cells

Mucosa-associated invariant T cells (MAIT)

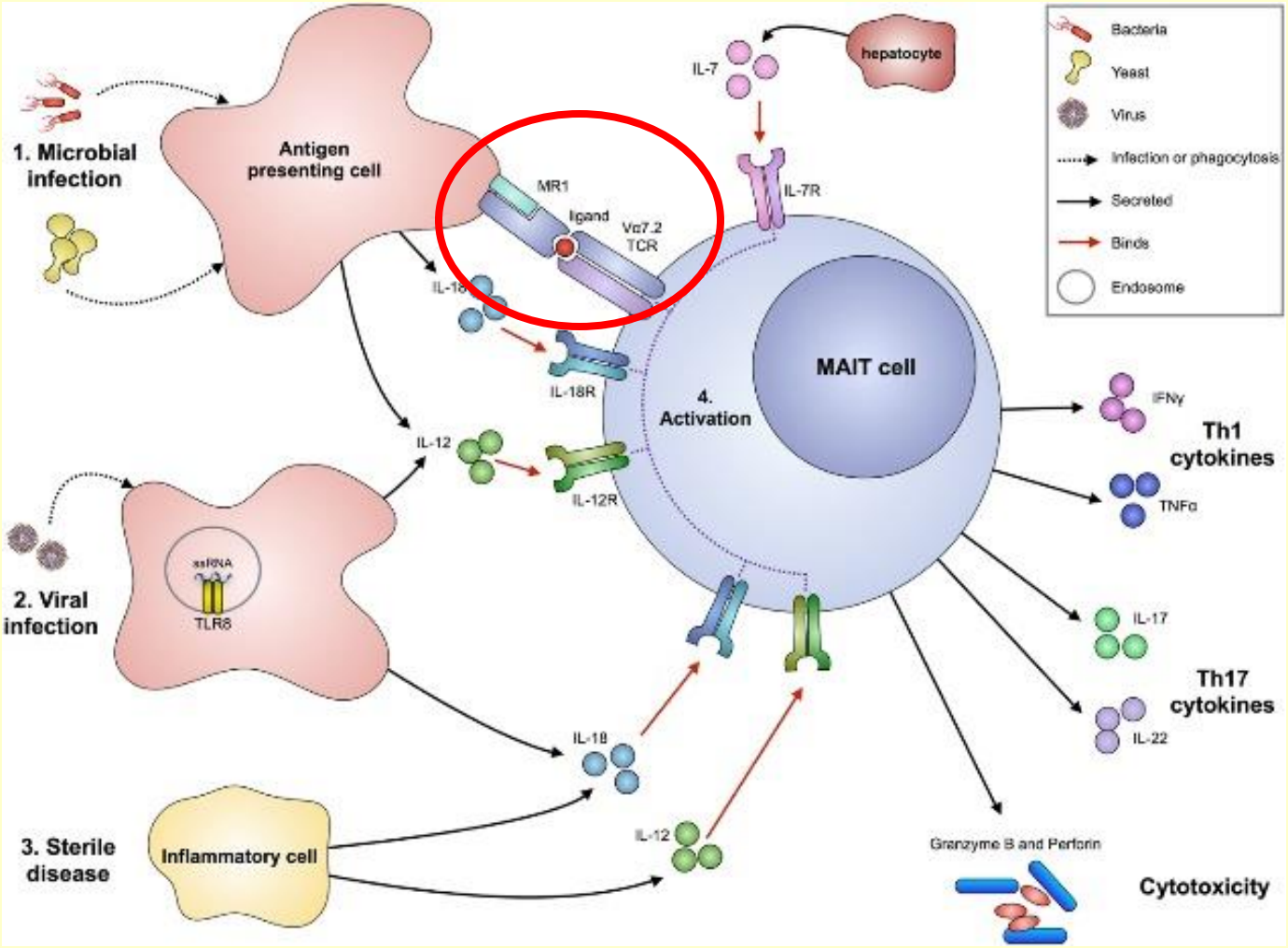


CD8+, CD26+, CD161+

1. MAIT cells arise from the thymus and are present predominantly in the gastrointestinal tract and associated organs such as MLNs and the liver.
2. In periphery by encountering the commensal flora, MAIT cells expand and acquire a memory phenotype.
3. They have antimicrobial function and help fight off bacterial infection by responding to infected cells and producing cytokines → Role in intestinal homeostasis.....
4. Innate sensors of infection as they accumulate early in infected tissues

http://dx.doi.org/10.1016/j.it.2011.02.005

Mucosa-associated invariant T cells (MAIT)



Mucosa-associated invariant T cells (MAIT)

- MAIT cells recognize MR1 and the associated microbial ligands on resident APCs, such as macrophages, dendritic cells or B cells, or directly on intestinal epithelial cells.
- In the absence of inflammation, MAIT cells participate in the control of the commensal flora or food-borne antigens by modulating APC function, or by regulating epithelial cell homeostasis and secretion of antimicrobial molecules.
- In case of bacterial invasion, however, the provision of the MR1-bound ligands to infected epithelial cells or APCs, in an inflammatory context (production of IL-18, IL-12 or IL-23, for which MAIT cells have receptors) induce production of IFN-γ by MAIT cells to prevent intracellular bacterial replication.
- Under certain conditions, MAIT cells can also secrete granzymes and other cytotoxic molecules to kill potential target cells, or IL-17 to activate innate immune cells such as neutrophils.

Delayed type hypersensitivity (DTH)

Type IV. hypersensitivity

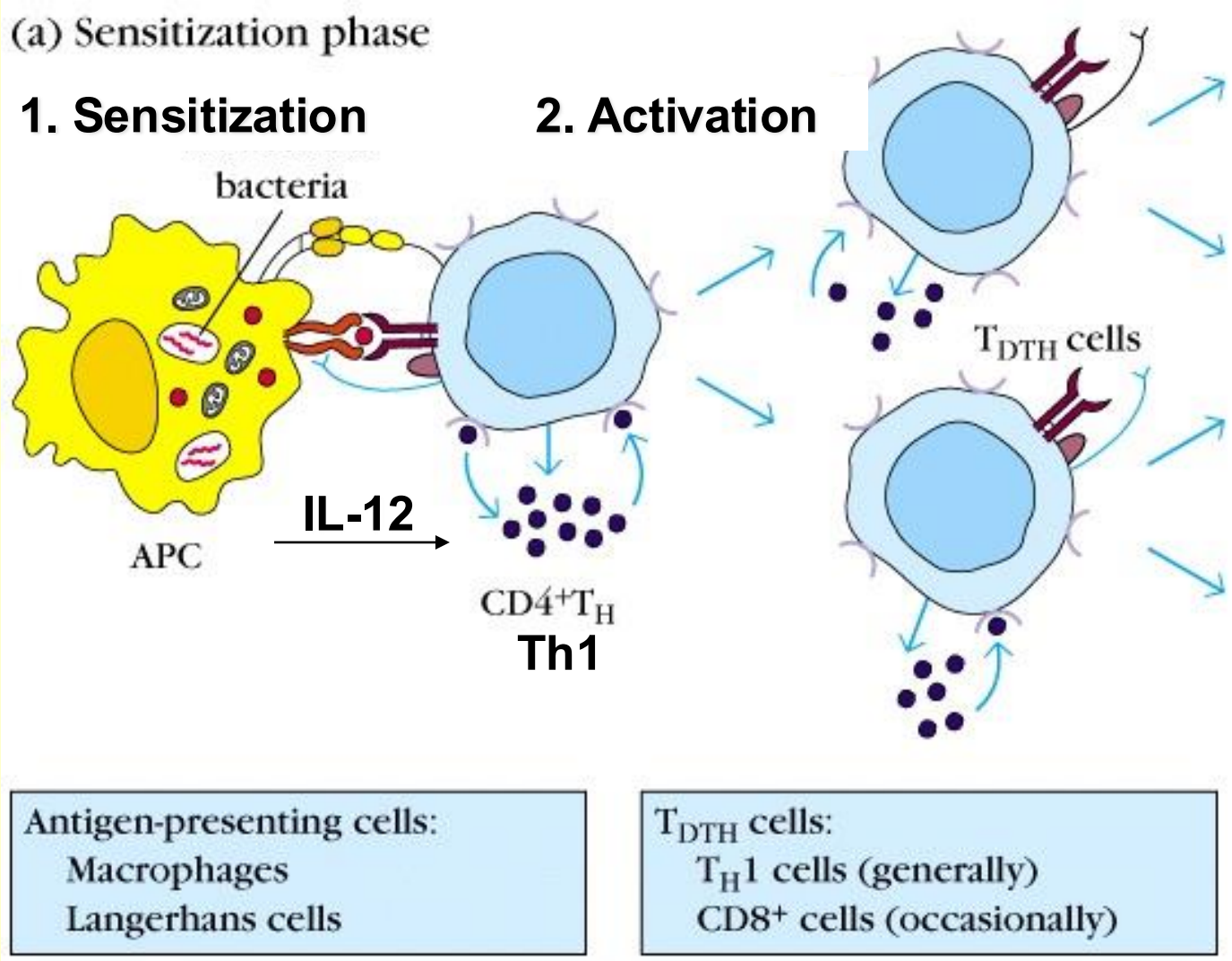
TABLE 14-3 INTRACELLULAR PATHOGENS AND CONTACT ANTIGENS THAT INDUCE DELAYED-TYPE HYPERSENSITIVITY

Intracellular bacteria	Intracellular viruses
Mycobacterium tuberculosis	Herpes simplex virus
Mycobacterium leprae	Variola (smallpox)
Listeria monocytogenes	Measles virus
Brucella abortus	Contact antigens
Intracellular fungi	Picrylchloride
Pneumocystis carinii	Hair dyes
Candida albicans	Nickel salts
Histoplasma capsulatum	Poison ivy
Cryptococcus neoformans	Poison oak
Intracellular parasites	
Leishmania sp.	

Self tissue antigens

Alloantigens (Transplantation)

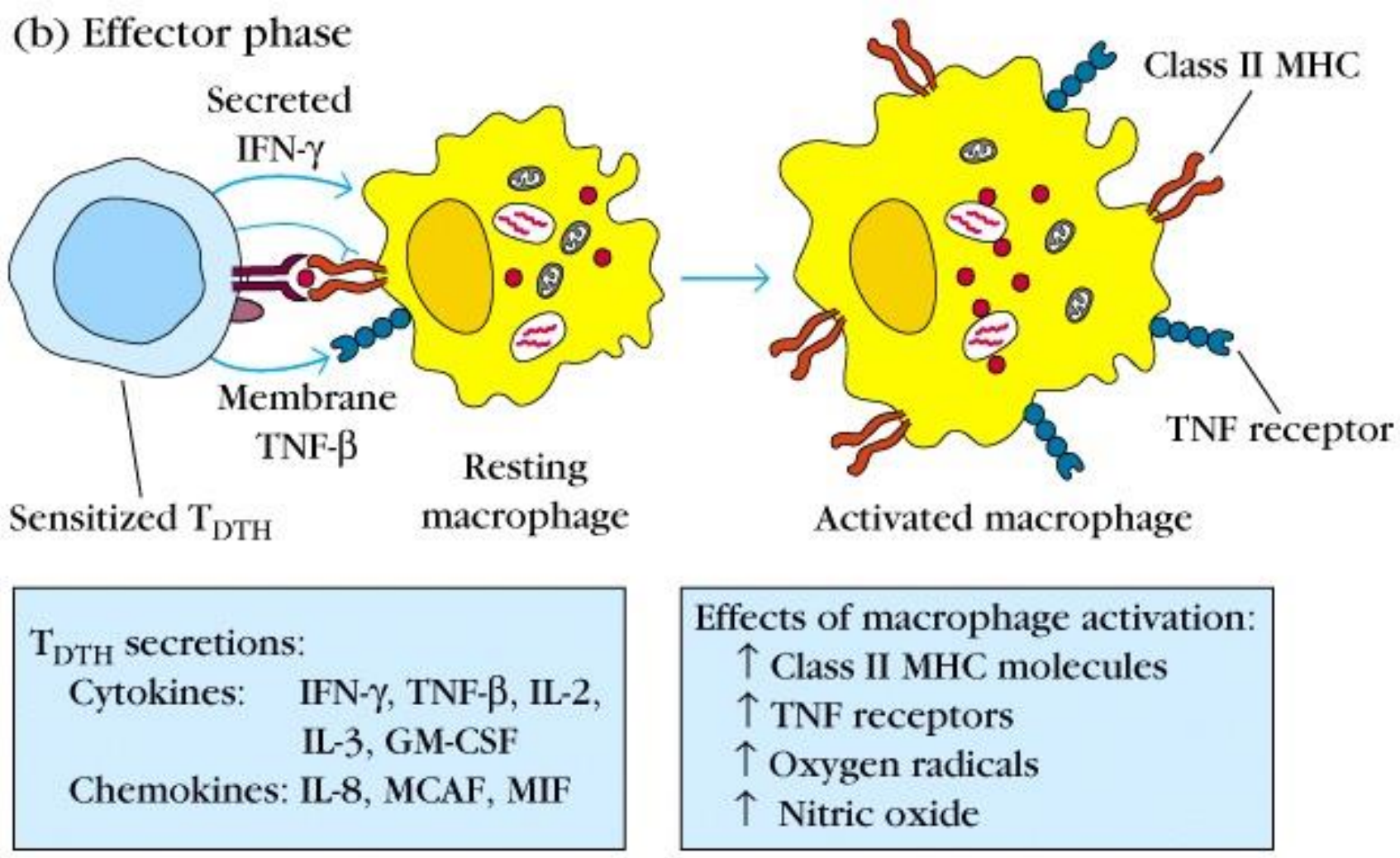
Phase 1 and 2 of DTH



1. Sensibilization: 1-2 weeks after the first antigen contact. APCs (Langerhans-cells, endothel cells or macrophages) produce IL-12 and induce Th1-cell differentiation.

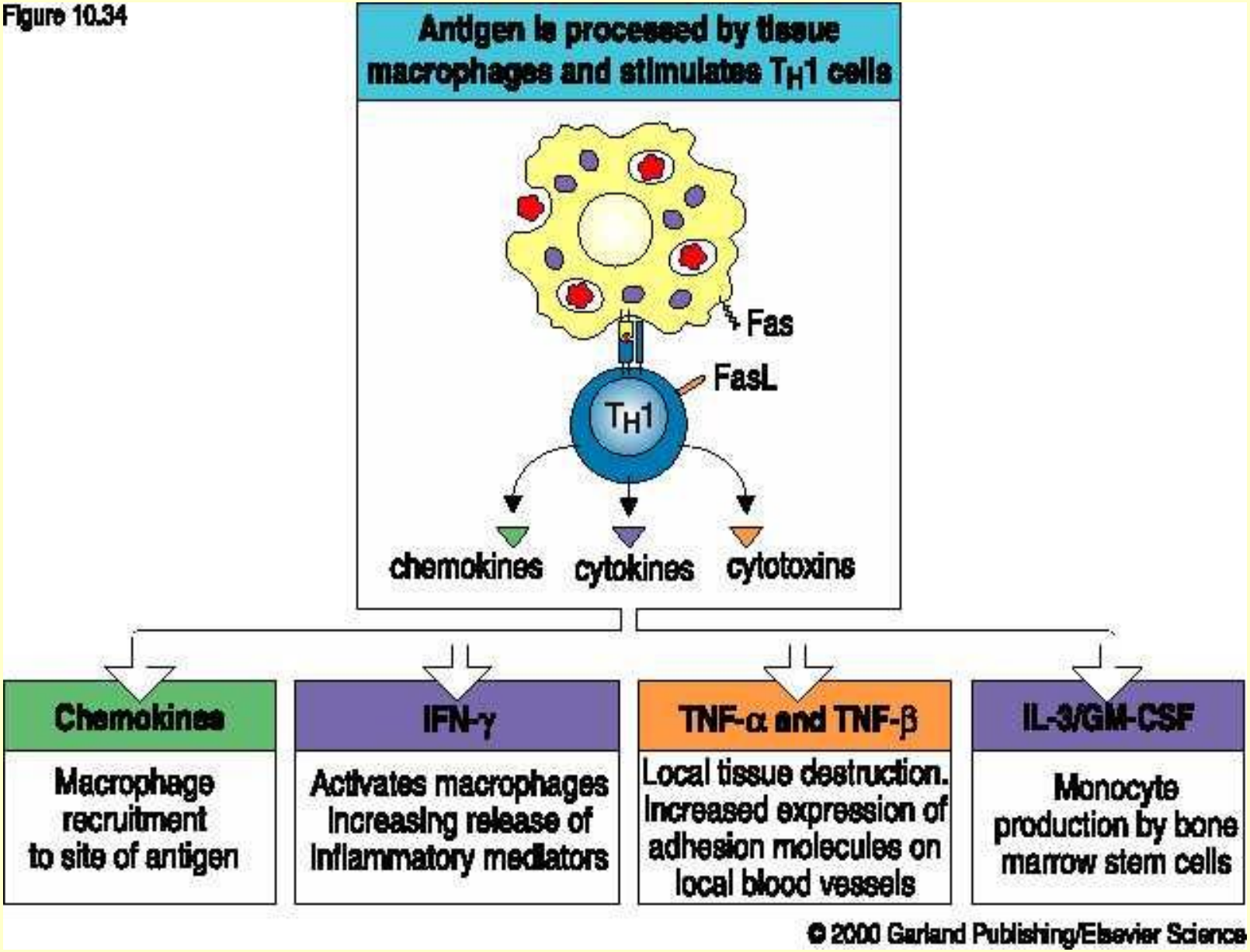
2. Activation: Th1-activation, proliferation, rarely CD8+ CTL-activation.

2. contact with the antigen



Effector phase: 2. antigen stimulus leads to Th1-cell activation, citokin secretion (24h), recruitment of macrophages and other non-specific inflammatory cells (48-72h). From the infiltrating cells only 5% is T cell, 95% is non-specific.

Type IV. hypersensitivity



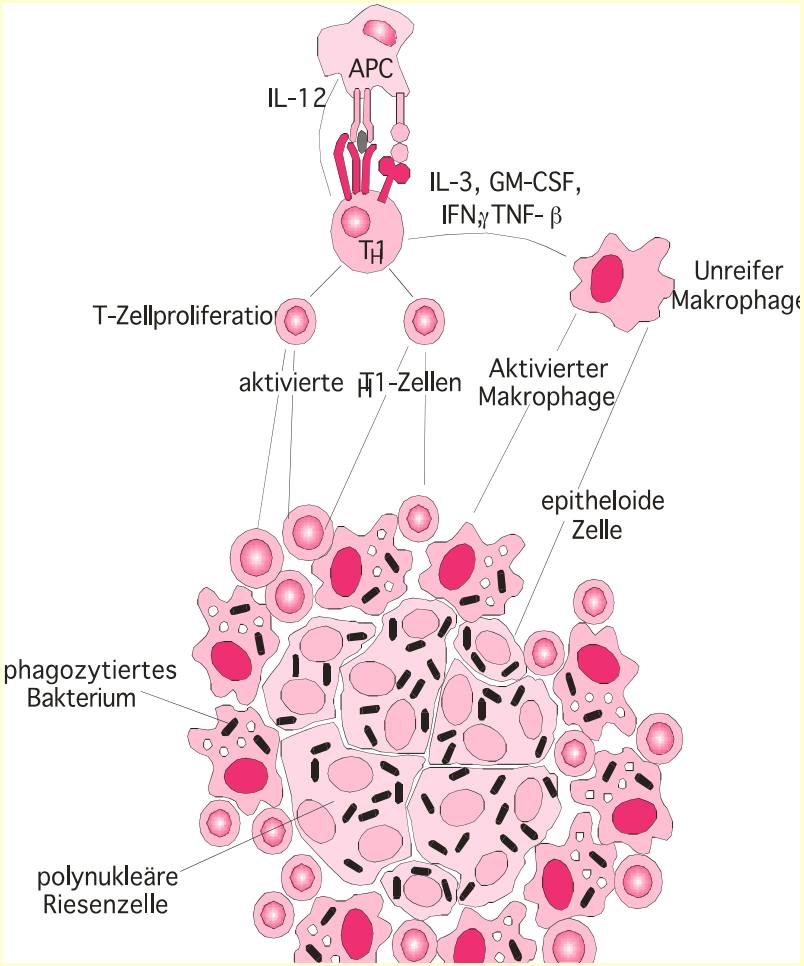
Stages of macrophage activation

Resting	**Activated**	**Hyperactivated**
---------->IFNgamma	---------->LPS, Immuncomplex double stranded RNA	
Phagocytosis	Antigen presentation	Tumor cell and parasite killing
Chemotaxis	Tumor cell binding	
Proliferation	decreased prolif.	No proliferation.
No cytotoxicity		**No APC**
MHC II -, O2 low	MHC II+, O2 high	MHCII -, O2high TNF,cytotoxic Protease secretion

4. phase of DTH

- **<u>Granulomatous-reaction</u>: if the intravesicular pathogen survives in the cells it induces a prolonged DTH response – <u>chronic infection</u>**
- **→ continous macrophage activation leads to citokin- and growth factor production and granuloma formation.**
- **Giant cells, epitheloid cells, tissue damage, necrosis, fibrosis.**

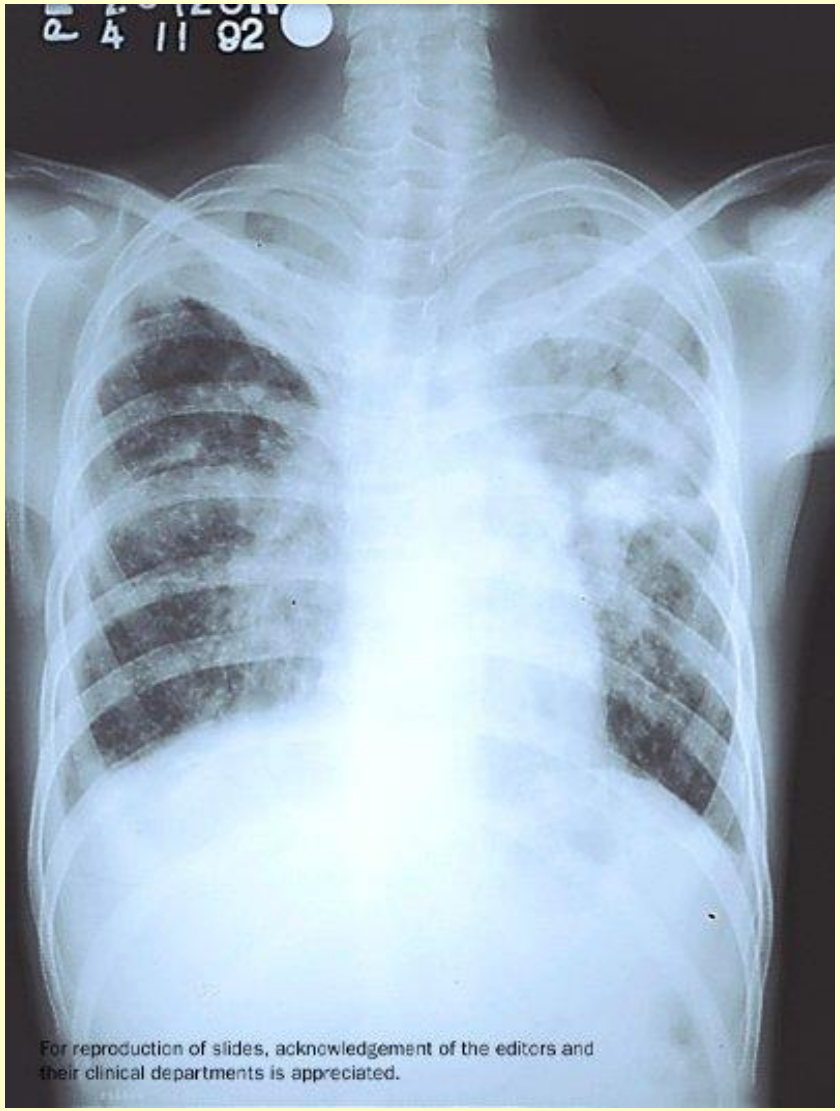
The structure of granulomas

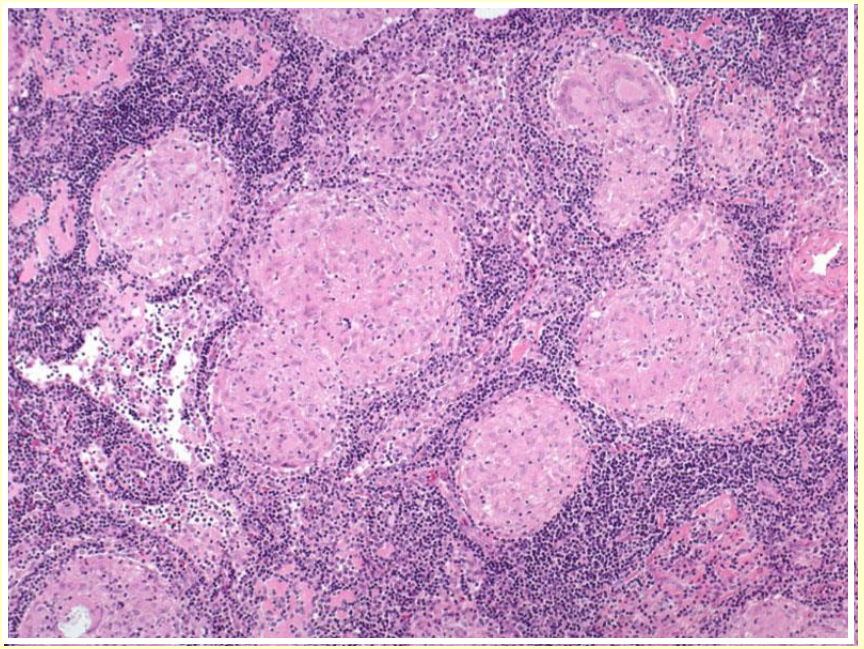


Diseases

- Infections: intracellular bacteria eg. *Mycobacterium tuberculosis, M. leprae;* Viruses: *Herpes simplex*
- Contact dermatitis, atopic ekzema
- Autoimmun diseases: Type 1 Diabetes Mellitus, Rheumatoid arthritis, Inflammatory bowel disease (IBD), Multiple sclerosis, Peripheral neuritis, Autoimmune myocarditis
- Transplant rejection: allogen tissue transplantation

Type IV. hypersensitivity –
Tuberculotic granulomas





Poison ivy (Toxicodendron)
Contact dermatitis