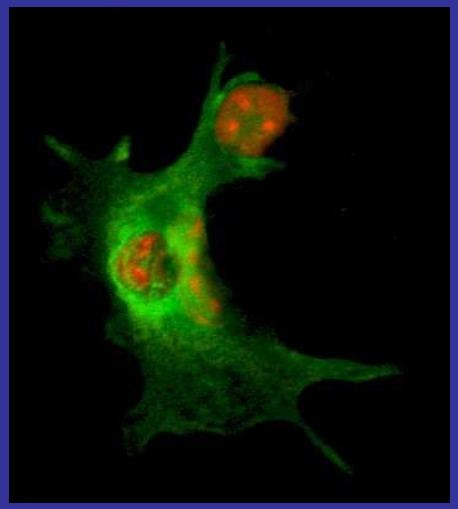

Dendritic cells:

Master switches of immunity

Prof. Dr. Diana Dudziak

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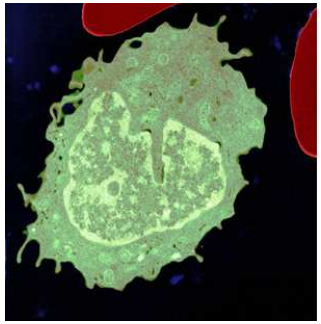
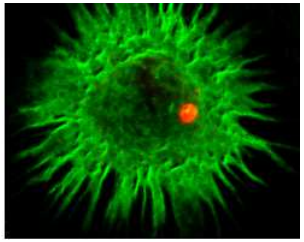
MEDIZINISCHE FAKULTÄT

Universitätsklinikum Erlangen

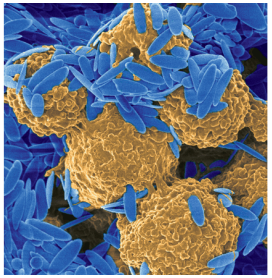
Antigen presenting cells



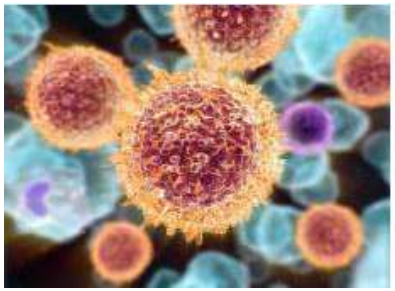
Monocytes



Dendritic cells

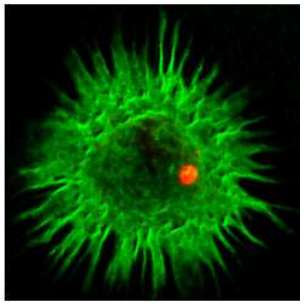


Makrophages



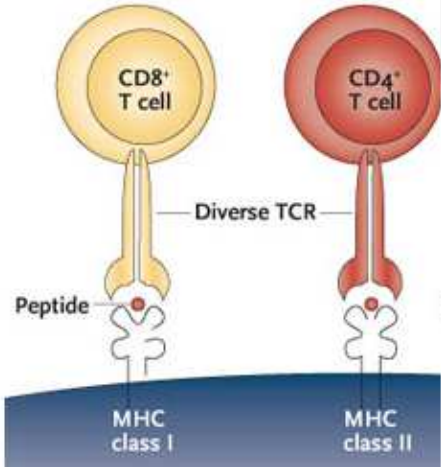
B cells

Dendritic cells (DCs)



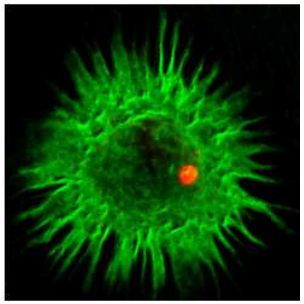
- tree like cytoplasm extensions (lat. *dendriticus*)
- 1868 discovery of Langerhans' cells in skin
- functional discovery of DCs by Ralph M. Steinman, 1973 (Nobel prize, 2011)
- very important antigen presenting cells
- bridge innate and acquired immunity

Dendritic cells (DCs)



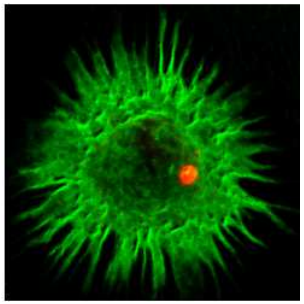
- tree like cytoplasm extensions (lat. *dendriticus*)
- 1868 discovery of Langerhans' cells in skin
- functional discovery of DCs by Ralph M. Steinman, 1973 (Nobel prize, 2011)
- very important antigen presenting cells
- bridge innate and acquired immunity
- needed for initiation of CD4 and CD8 T cell responses
- but also needed for maintenance of peripheral tolerance

Dendritic cells (DCs)



1. Localization
2. Antigen uptake and antigen processing
3. DC maturation and migration
4. Antigen presentation
5. T cell activation
6. What makes DCs so complicated?

Dendritic cells (DCs)



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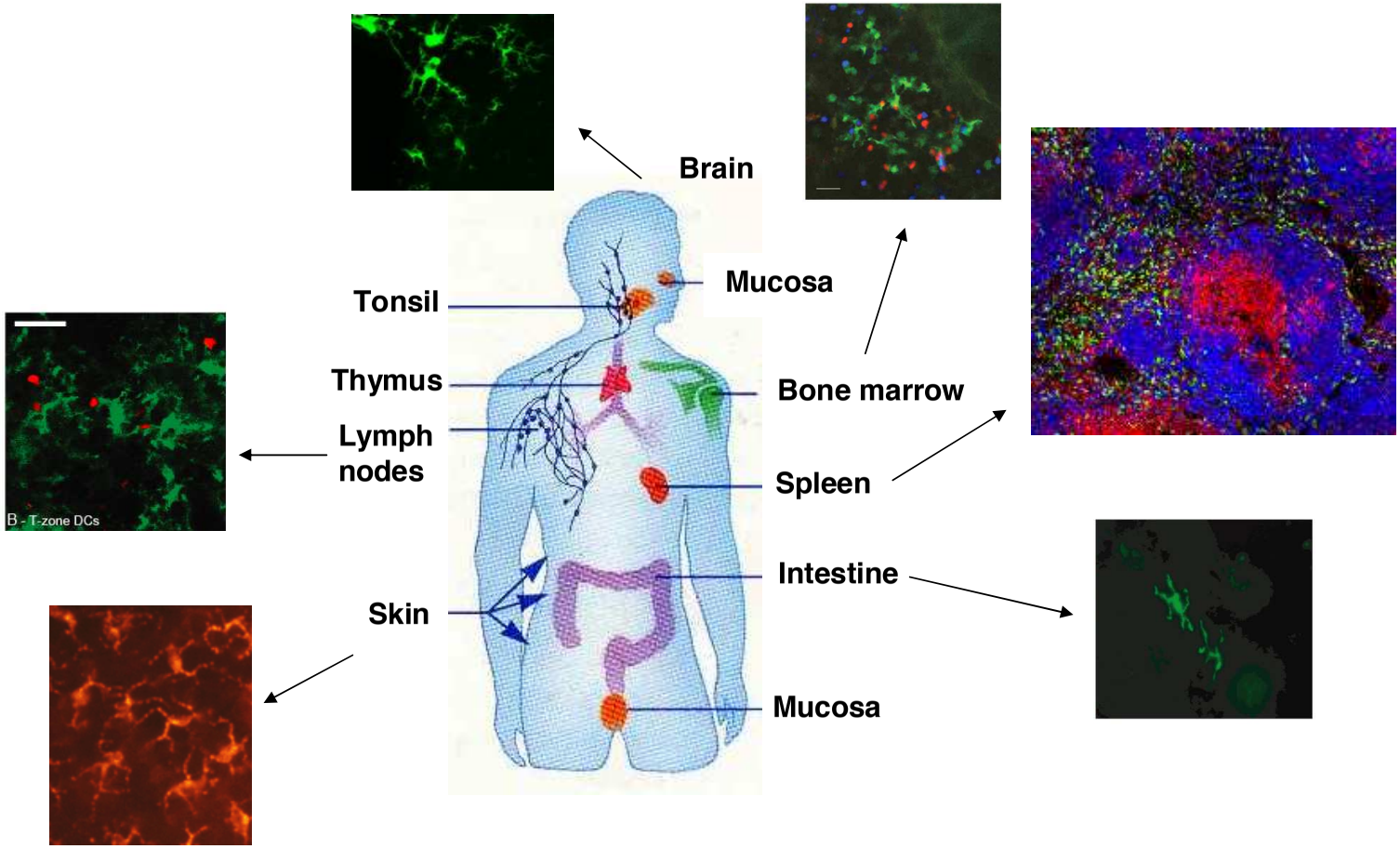
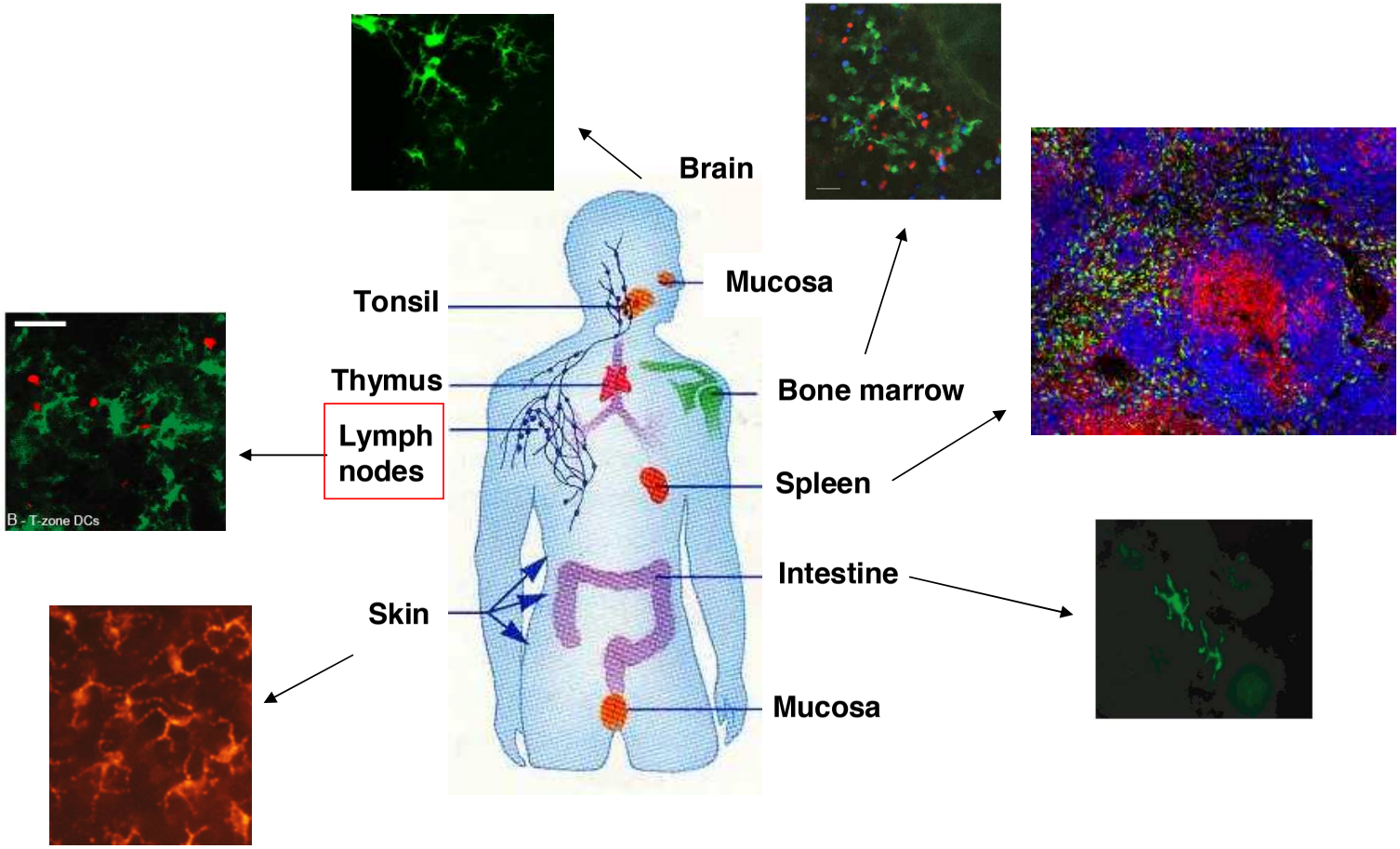
Localization of dendritic cells



Localization of dendritic cells

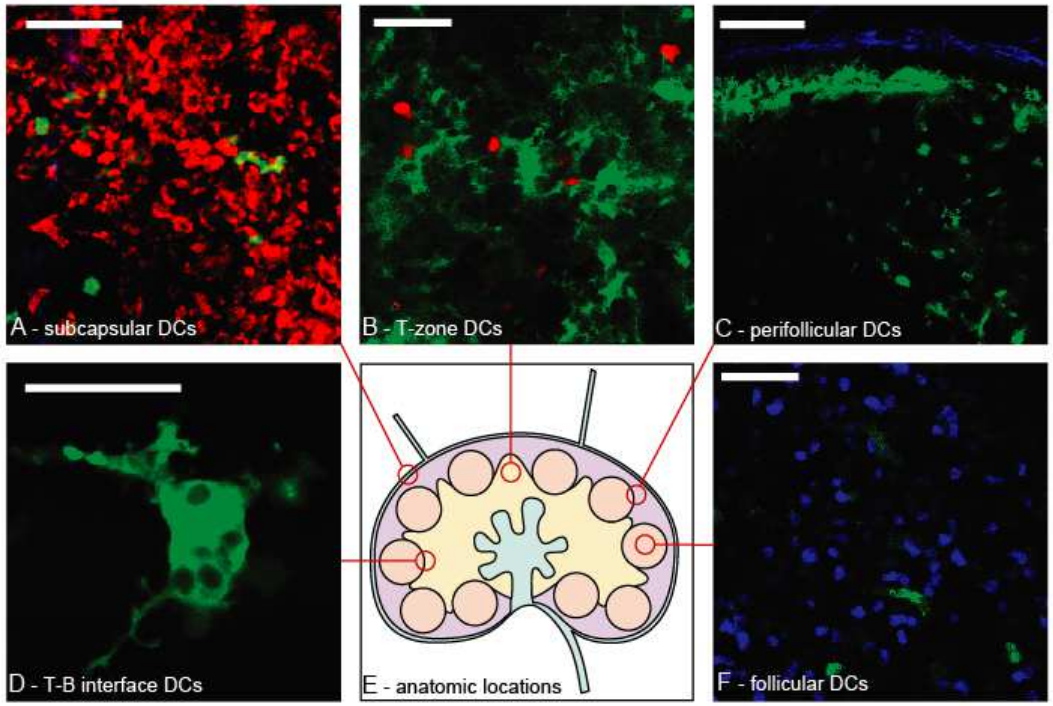


Differential localization and activity of dendritic cells in lymph nodes

C57BL/6 transgenic
CD11c Promoter::
EYFP mouse

Green DCs

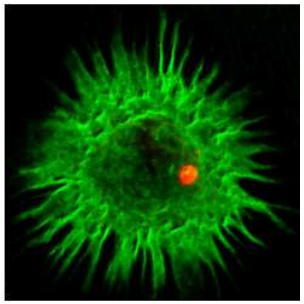
Intravital
2-Photon
microscopy



Velocity: B Zone >Subcapsular DCs > Perifollicular > T Zone

Lindquist et al., Nat. Immunol. (2004); Shakhar et al., 2005

Dendritic cells (DCs)



1. Localization
2. **Antigen uptake** and antigen processing

   (classical / alternative processing)
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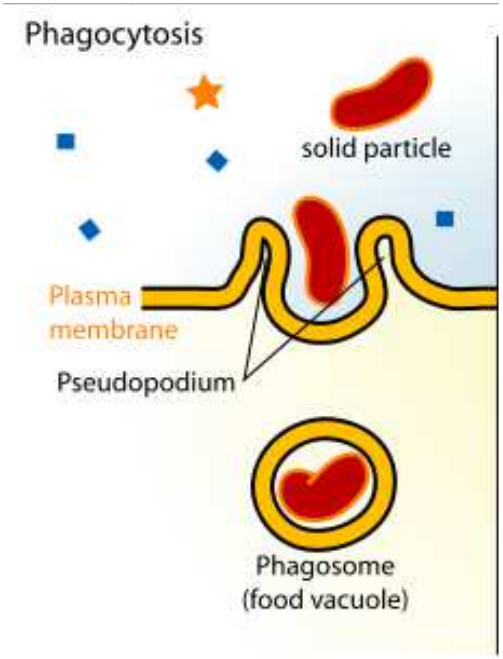
Antigens

- bacterial proteins
- viral proteins
- tumor material
- apoptotic/necrotic material
- immune complexes (IgG-antigen)

- lipids

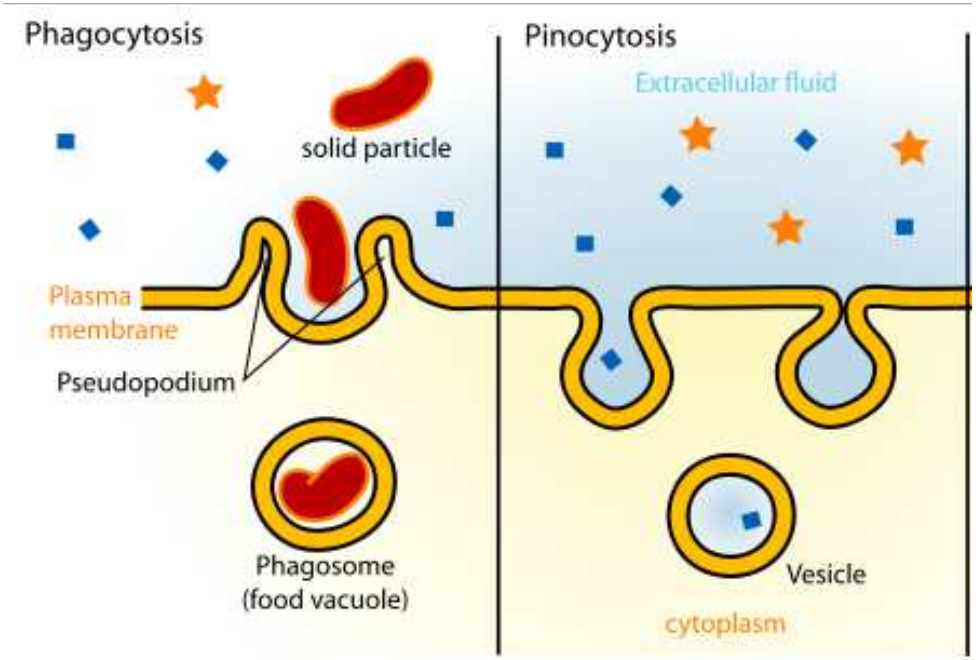
Antigen uptake



<u>Phagocytosis:</u>

- Particle (> Ø 1 μ m)
- membrane extrusions (pseudopod extension),
- Actin assembling (CDC42, Rac)

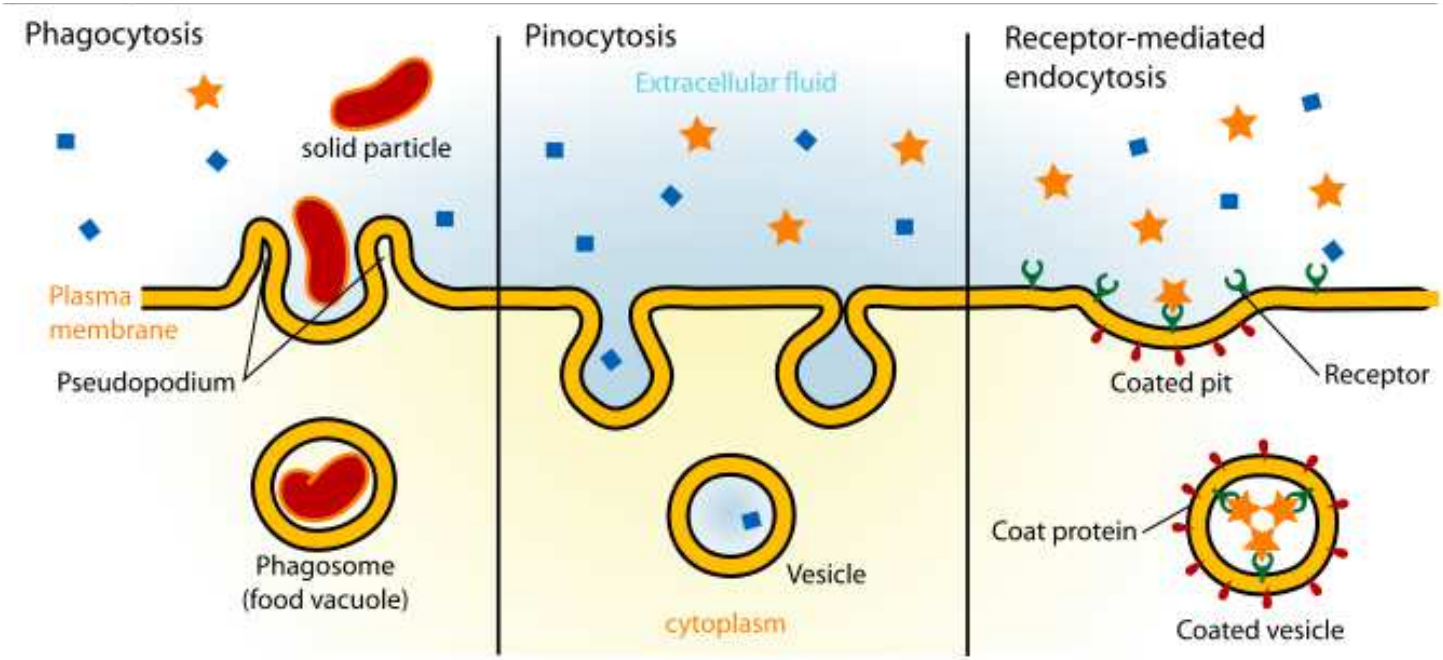
Antigen uptake



Macropinozytosis:

- constitutive process
- actin-mediated membrane invagination
- uptake of extracellular fluids and soluble antigens

Antigen uptake

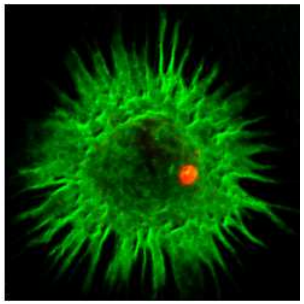


<u>Receptor-mediated endocytosis:</u>

- uptake into clathrin-coated vesicles
- ‘coated pits’
- function of caveolin-coated vesicles unknown

Additional information:
Trombetta and Mellman
Annu.Rev.Immunol. 2005

Dendritic cells (DCs)



1. Localization
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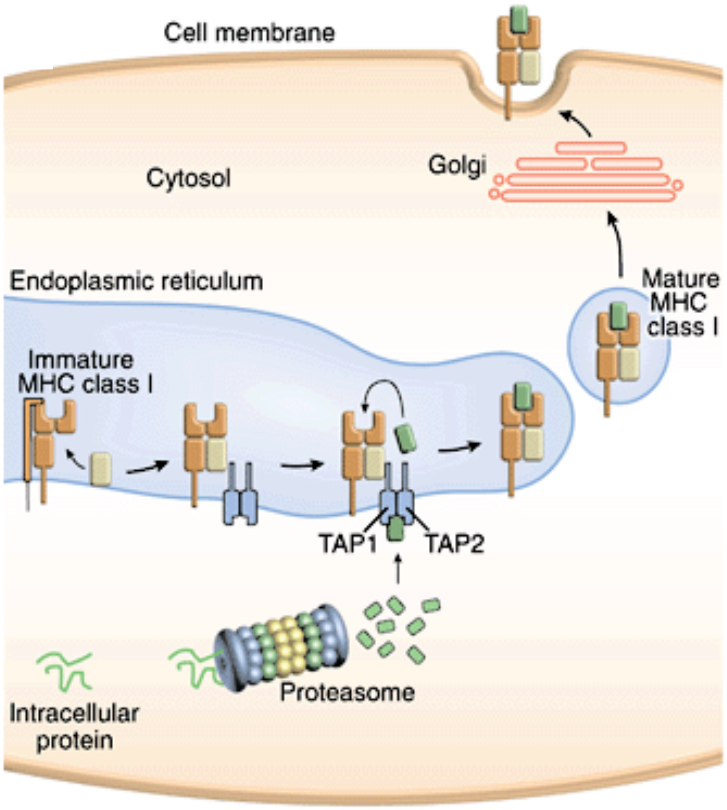
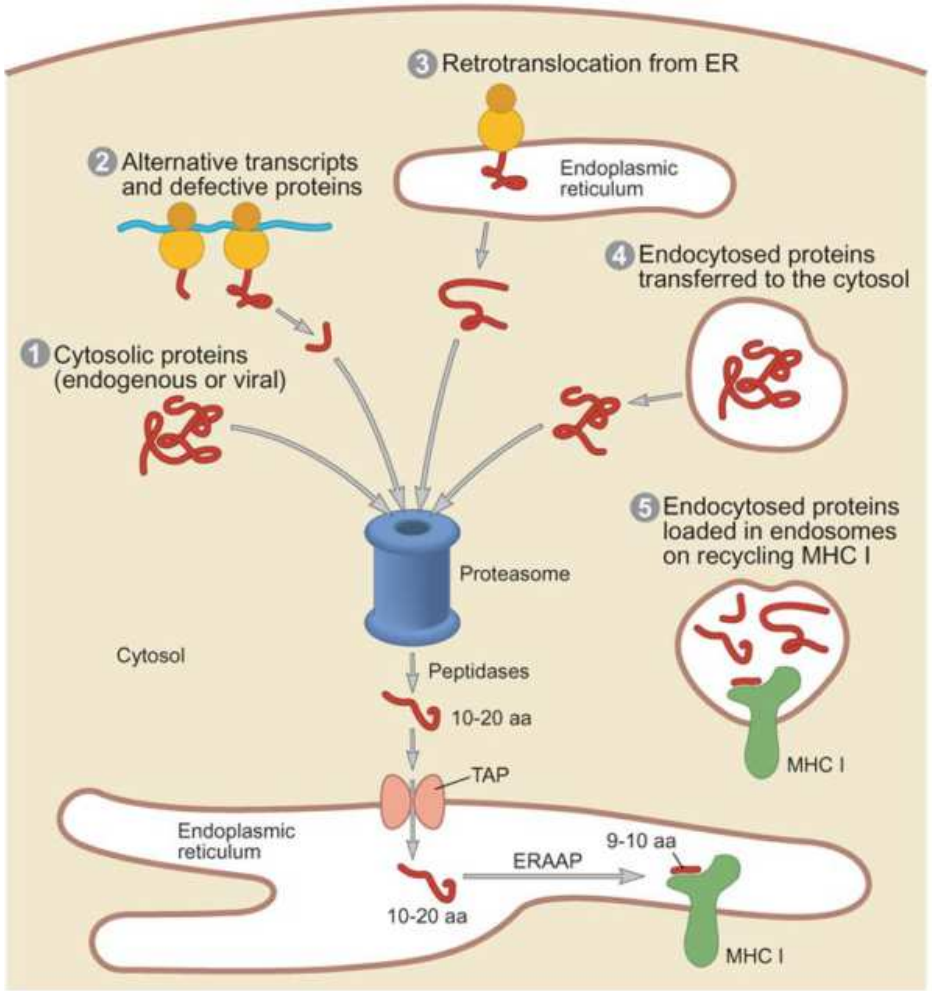
Antigen processing in the context of MHCI



- degradation of cellular or viral antigens in the proteasom
- TAP-transporter transports antigen into endoplasmatic reticulum (ER)
- antigen-loading of MHC-class I molecules
- transport of peptid-MHCI-complexes via Golgi network to the cell surface

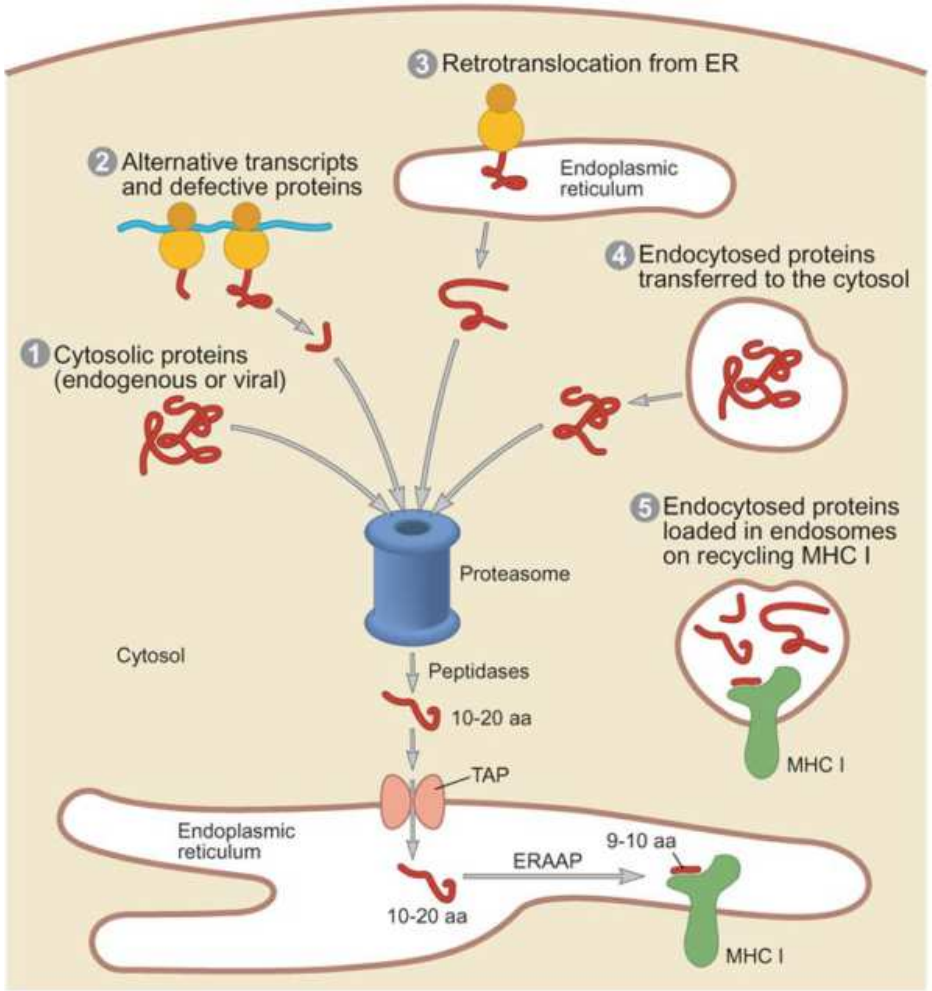
Antigen sources for MHC class I



1. cytosolic proteins
2. alternative transcripts and defective proteins (DRiPs)
3. proteins derived from ER
4. endocytosed proteins transferred into the cytoplasm
5. endocytosed proteins loaded in endosomes to recycling MHCI

Trombetta and Mellman
Annu. Rev. Immunol., 2005

Antigen sources for MHC class I

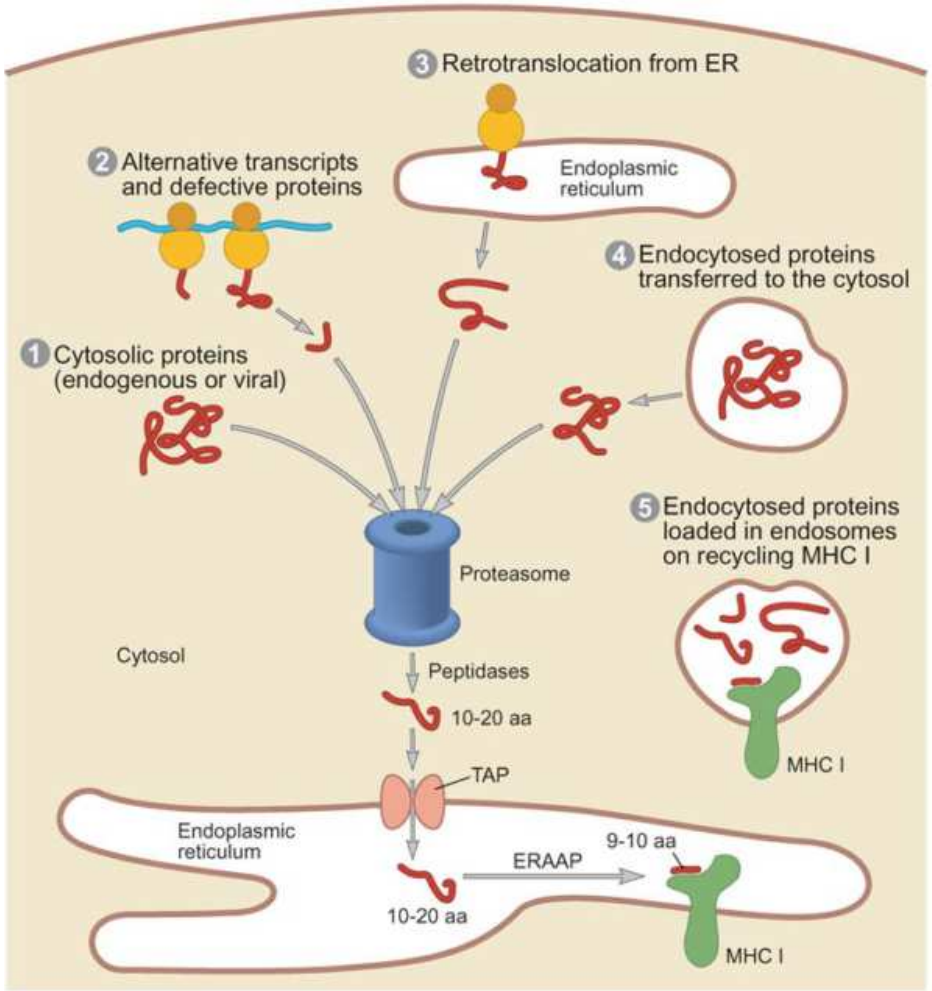


DRiPs:

1. (***d**efective **ri**bosomal **p**roducts)*
2. short lived proteins,
   non-complete proteins,
   wrongly folded
3. self and viral peptides
4. intracellularly produced

Yewdell, JI, 1996
Trombetta and Mellman
Annu. Rev. Immunol., 2005

Antigen sources for MHC class I

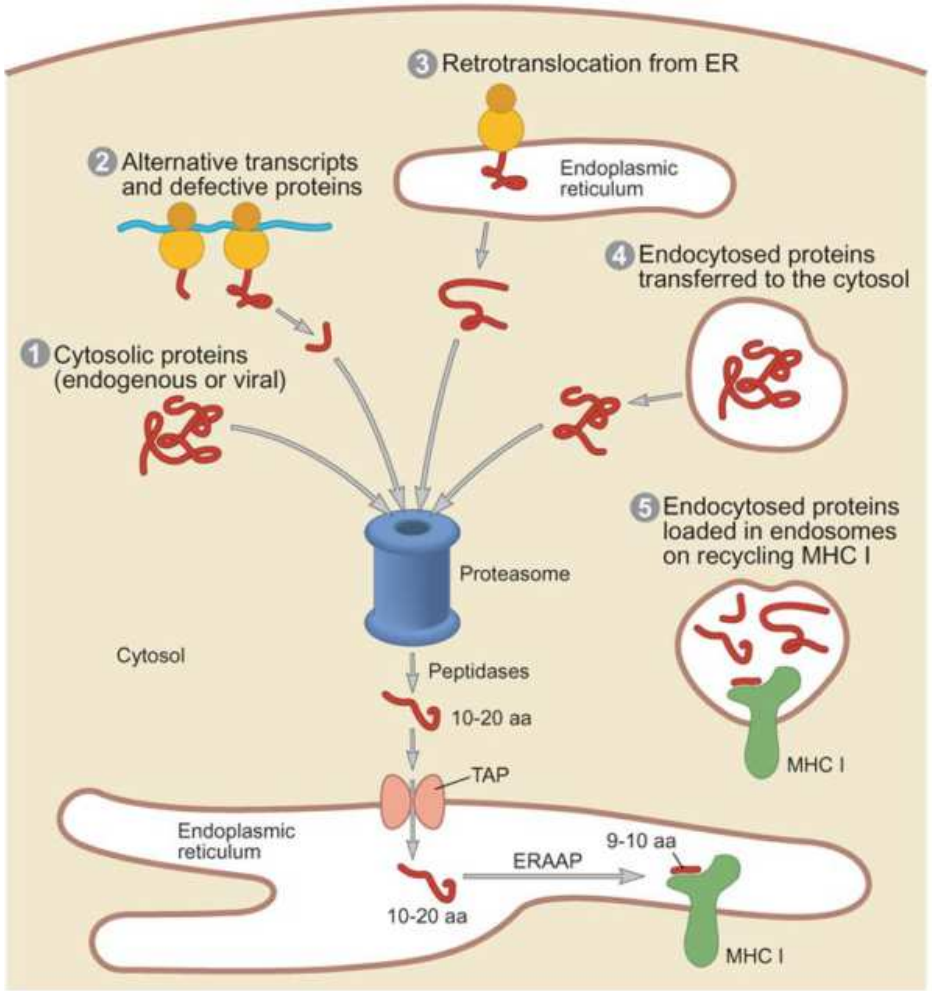


Labeling of proteins that need to be degraded:

1. Ubiquitin (Ub), 76 aa, 8 kDa
2. E1, E2, E3 Ub-ligases involved in ubiquitination of Lysine residues of molecules that need to be degraded

Trombetta and Mellman
Annu. Rev. Immunol., 2005

Antigen sources for MHC class I



Proteasome:

1. 2MDa, 15nm long, 11nm, 30.000 per cell
2. central garbage can (20 S-core), two 19 S-capping complexes
3. <u>20 S Core:</u> Ring of each 7α -, and β-subunits (αββα), multicatalytic proteinase
4. <u>ß1i (LMP2)</u>
   - low PGPH-activity (cleavage after acidic aa)

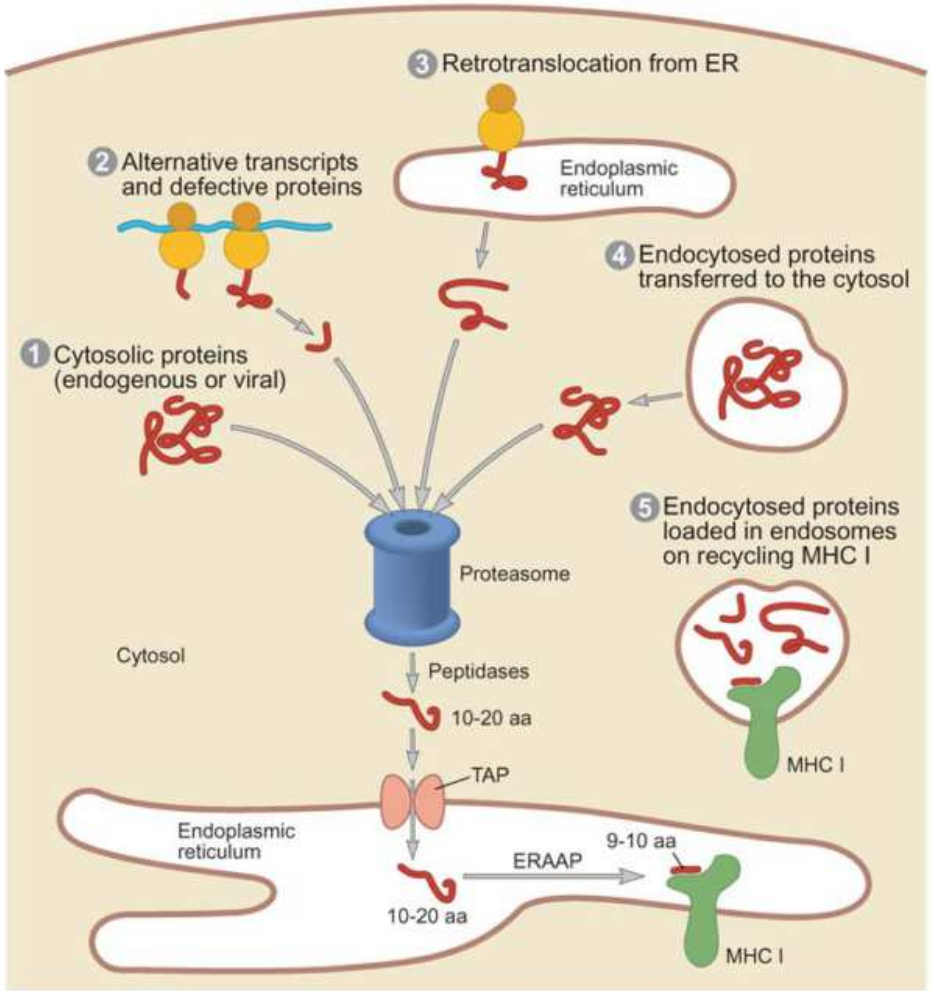
   <u>ß2i (MECL-1)</u>
   - tryptic activity (cleavage after alkaline aa)

   <u>ß5i (LMP7)</u>
   - chymotryptic activity (cleavage after hydrophobic aa)

PGPH: peptidylglutamyl peptide hydrolyzing

Trombetta and Mellman
Annu. Rev. Immunol., 2005

Antigen sources for MHC class I

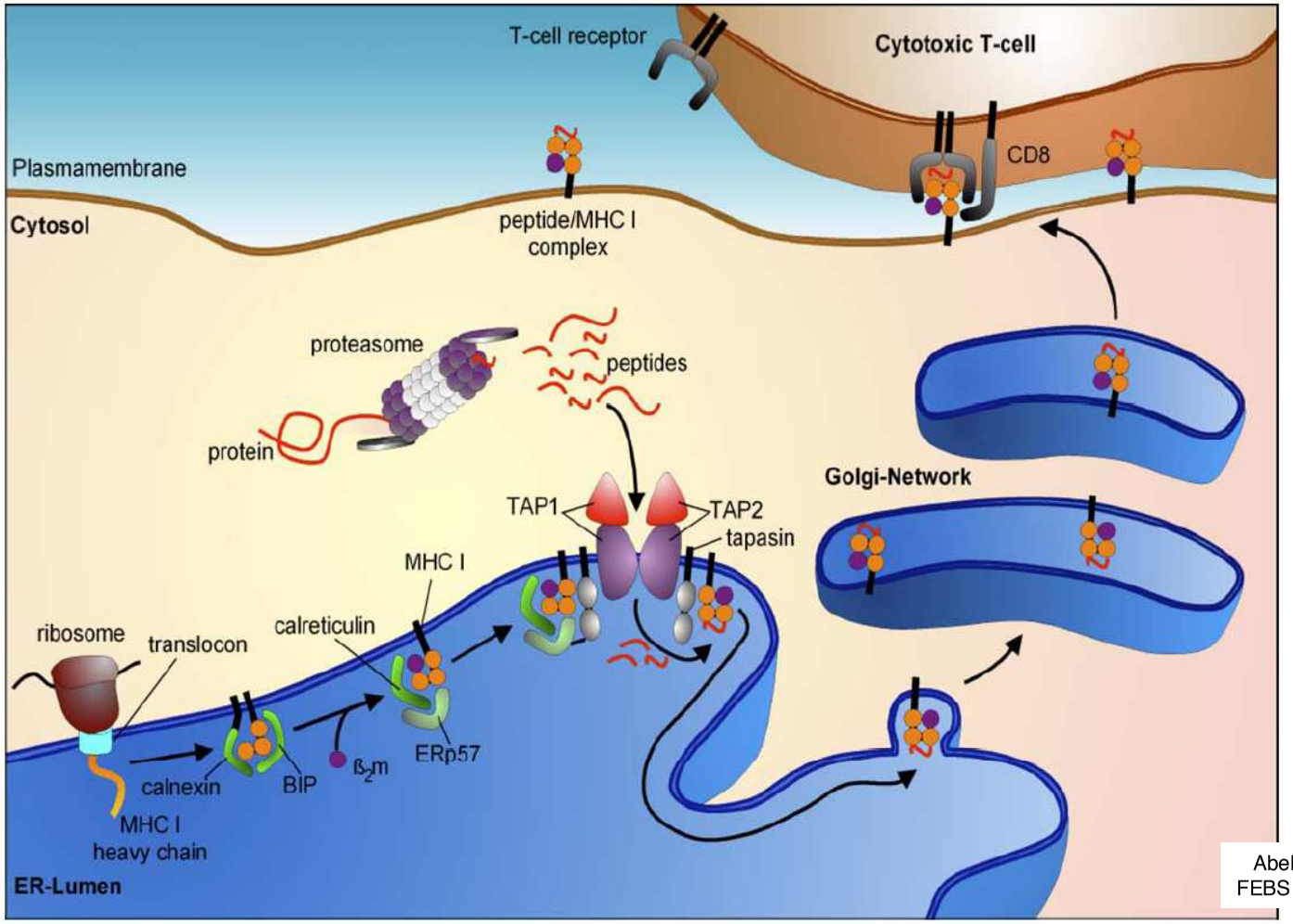


Proteasome:

1. ubiquitinated protein binds to 19S-complex
2. Ub will be cleaved, ATP-dependent
3. proteins unfold
4. proteases on the active centers of the β-subunits cleave proteins in 10-20 aa peptides
5. further cleavage by cytosolic peptidases

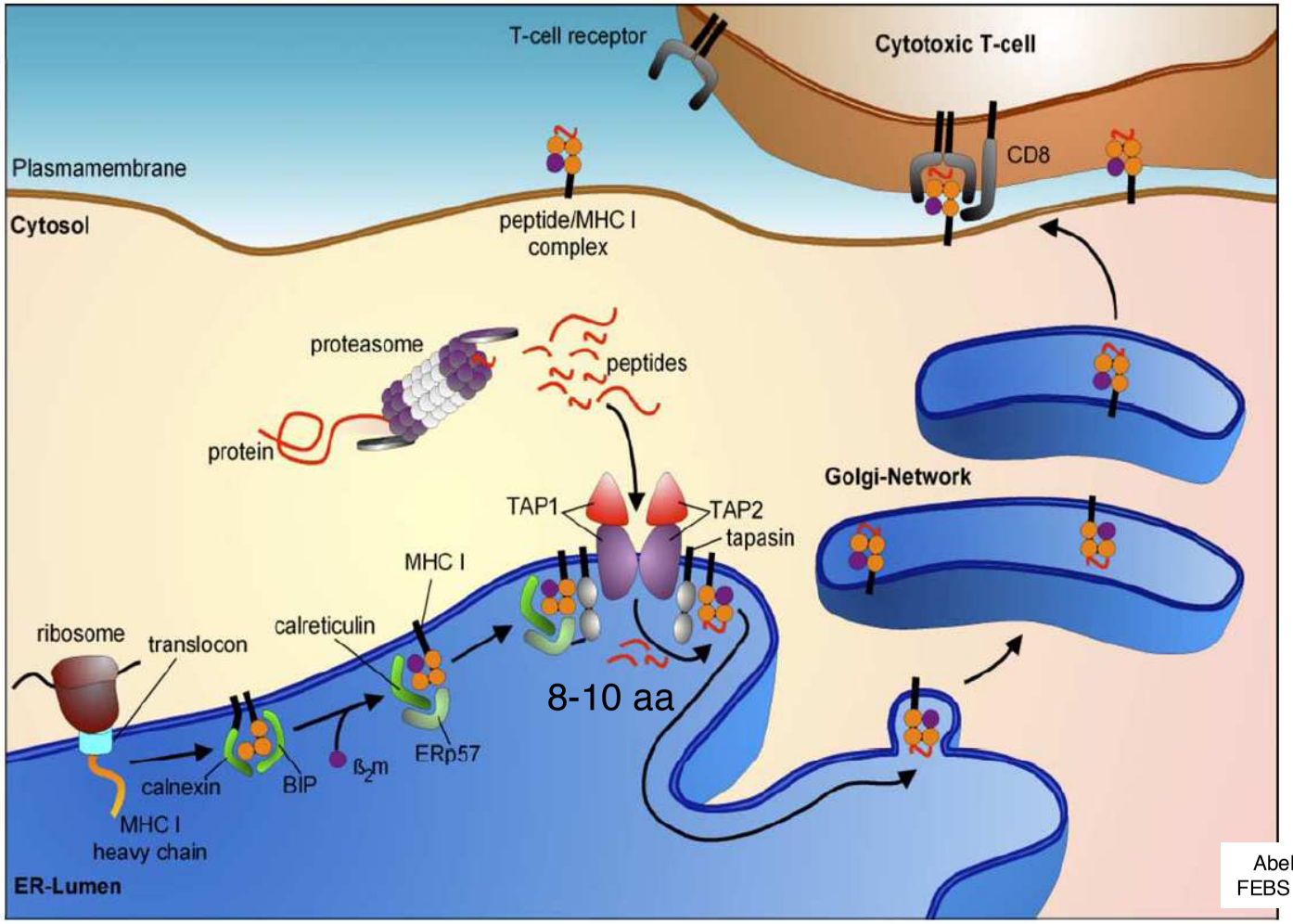
Trombetta and Mellman
Annu. Rev. Immunol., 2005

MHC class I antigen processing



Abele and Tampé
FEBS Letters, 2006

MHC class I antigen processing



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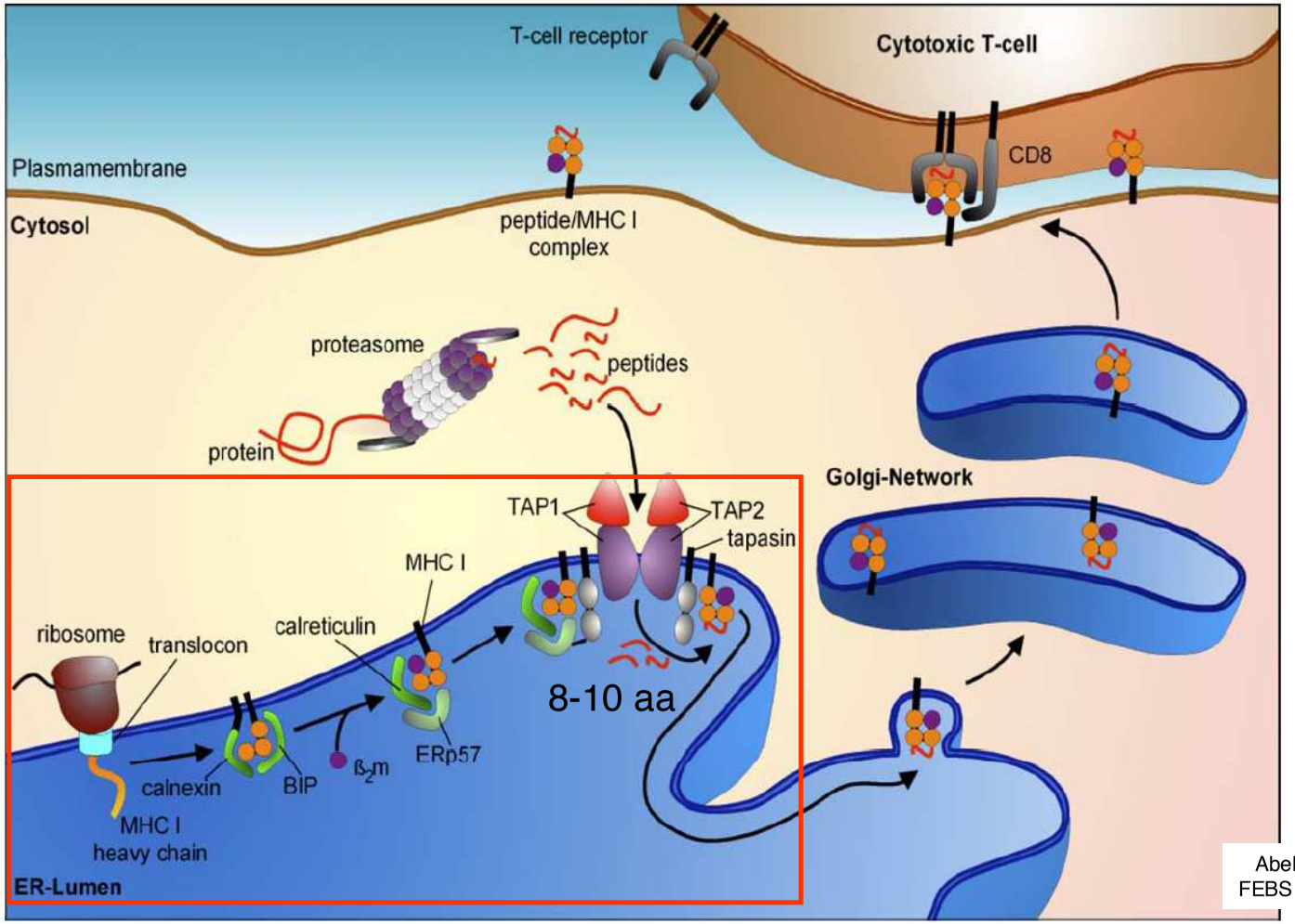
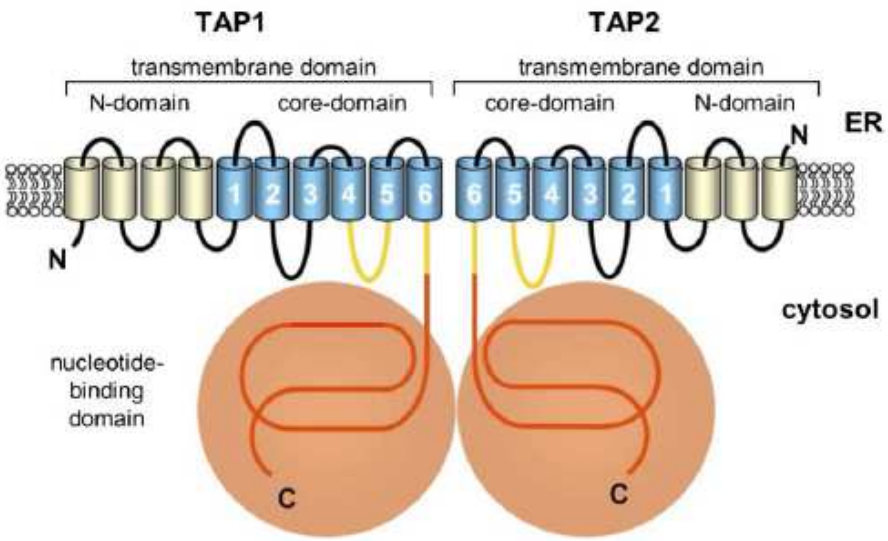


Abele and Tampé
FEBS Letters, 2006

TAP transporter



- TAP transporter (**T**ransporter associated with **A**ntigen **P**rocessing)

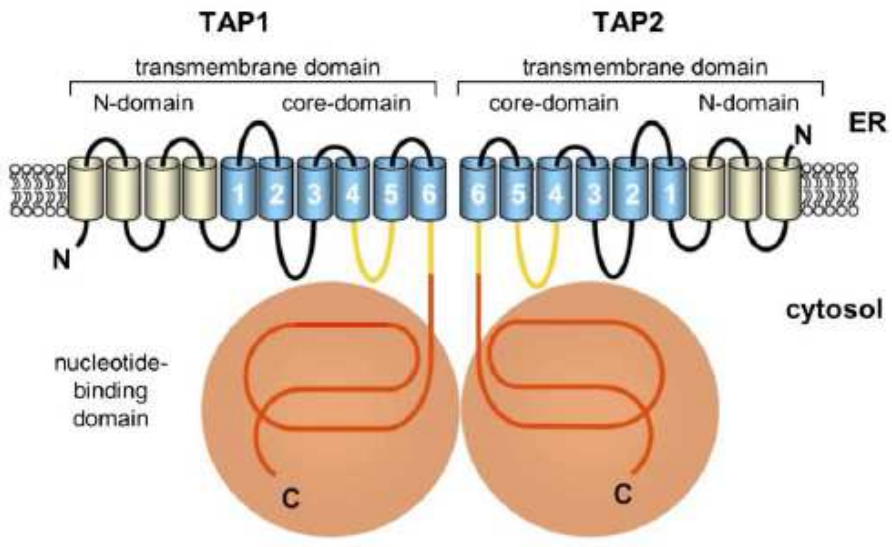
  with **A**TP-**b**inding **c**assette (ABC-transporter)

- transport of cleaved protein fragments/peptides from cytosol into ER

- localized in ER and cis-Golgi)

Abele and Tampel
FEBS Letters, 2006

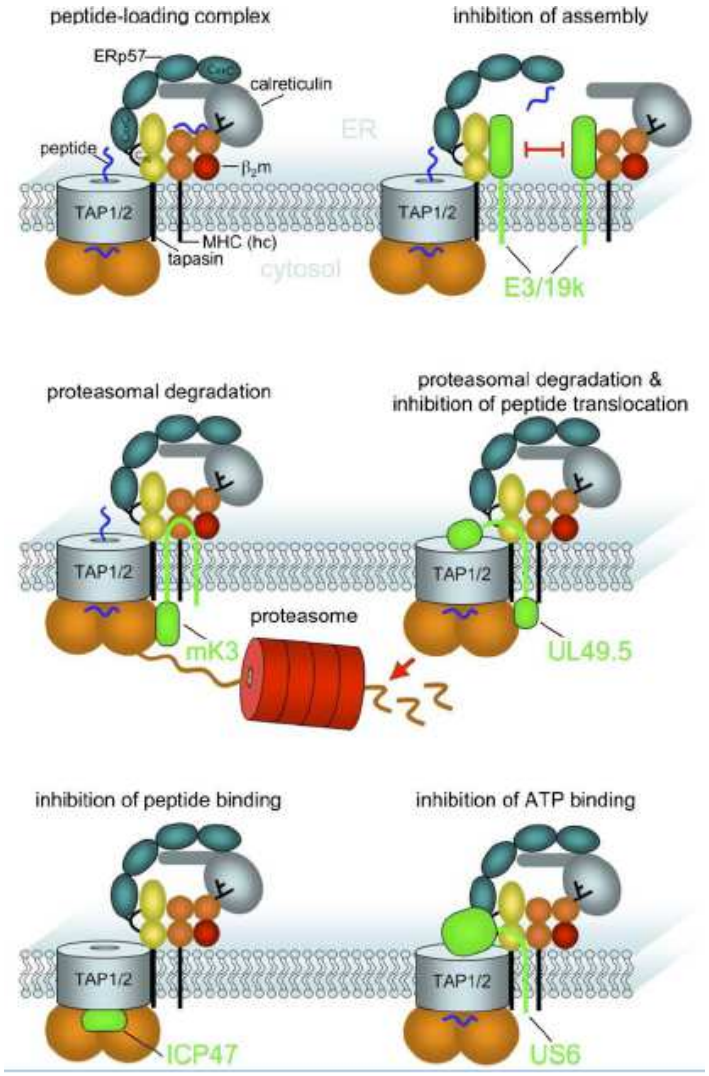
TAP transporter



- upregulated by IFN-γ, stabilization of the complex
- in tumors low TAP activity
- heterodimer of TAP1 (748aa) and TAP2 (686aa)
    - TM-domain with peptide binding side
    - cytosolic nucleotide-binding domain (NBD) –
- transport of 8-16 and 8-12 aa

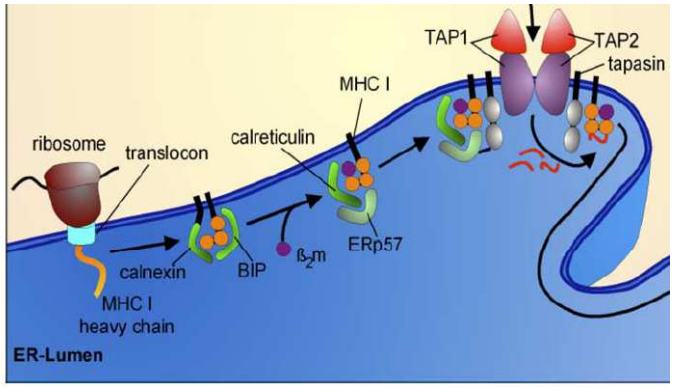
Abele and Tampel
FEBS Letters, 2006

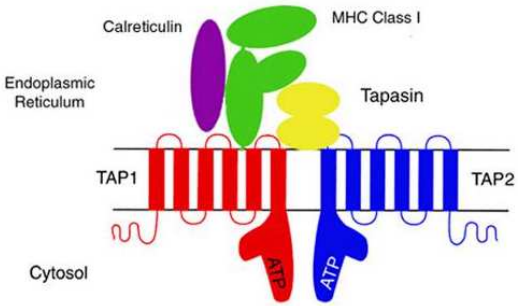
Viral Interference with TAP transporter



- Modulation of TAP function by viral factors. Upper panel: The structural organization of the peptide-loading complex (PLC) is illustrated as a model. ERp57 is covalently linked to tapasin via a disulfide bond (C95 of tapasin to the CxxC motif of the oxidoreductase).
- Within the ER quality control, calreticulin recognizes the N-core glycosylation of the MHC I heavy chain (hc) and forms additional contacts with ERp57. For simplicity, only one of four TAP-associated sub-complexes (tapasin, MHC heavy chain, b2m, ERp57 and calreticulin) is shown.
- The **E19 protein of adenovirus E3 disturbs** the **interaction of MHC class I** molecules **with** the pre-assembled **TAP–tapasin–ERp57** complex which impairs efficient peptide loading of MHC class I.
- Middle panel: The **mK3 protein of murine c-herpesvirus-68 binds** directly to **TAP** and **induces polyubiquitylation** and subsequently proteasomal **degradation**.
- The interaction of **UL49.5 of the bovine herpes virus drives TAP to proteasomal degradation** and arrests TAP in a transport-incompetent conformation, in which binding of ATP and peptide is not affected.
- Lower panel: **ICP47** of the **herpes simplex virus inhibits peptide binding** from the cytosolic site of **TAP**.
- The association of **US6 of the human cytomegalovirus** to the ER-luminal transmembrane core of TAP **blocks ATP binding** to the cytosolic peptide loading complex.

Peptide loading complex (PLC)



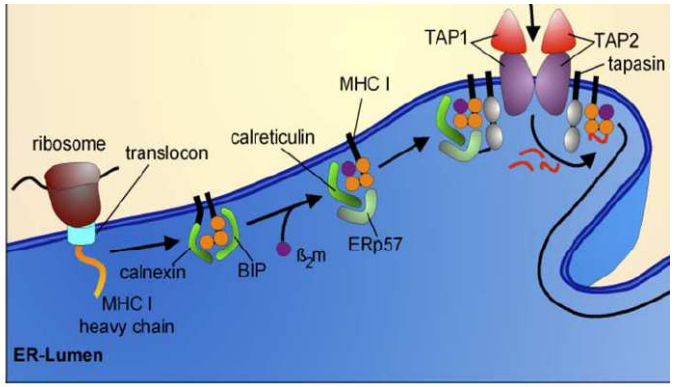


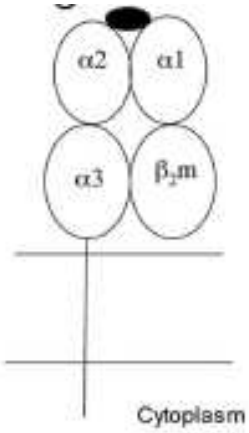
-TAP$^{-/-}$ mice - peptide transport into ER blocked

- no (few) MHC-I-peptid-complexes on the surface

- multi-component-peptide-loading-complex (PLC)
  - TAP heterodimer
  - 4 molecules tapasin (TAP-binding protein= TAP-BP) and
  - 4 molecules MHC-class-I
  - lectin-like chaperons: Calreticulin, Calnexin, Oxidoreductase ERp57

β2 microglobulin



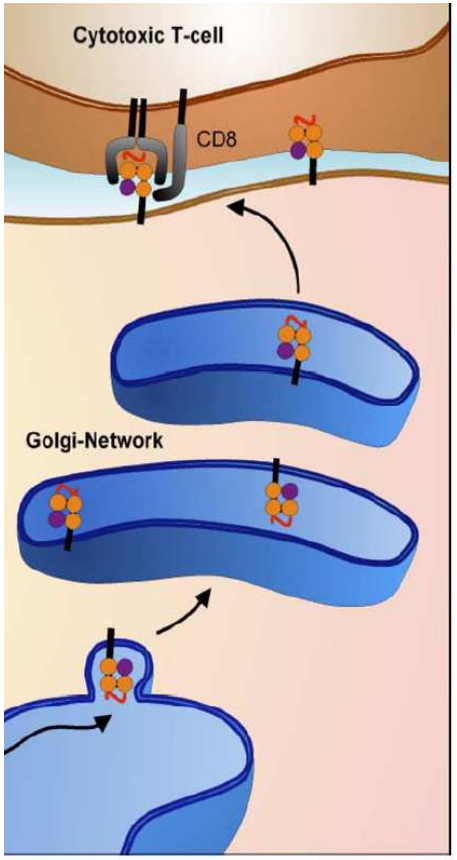


β2 microglobulin

- essential for transport of MHCI to the cell surface
- stabilizes peptides in MHCI-binding grove
- is β2M missing - low to no MHCI molecules on the surface
- development of CD8 T cells distracted
- β2m not polymorph
- laterally to α3 subunits of MHC-I (also in CD1, non-classical MHC-molecules),
- no TM-domain

Abele and Tampé
FEBS Letters, 2006

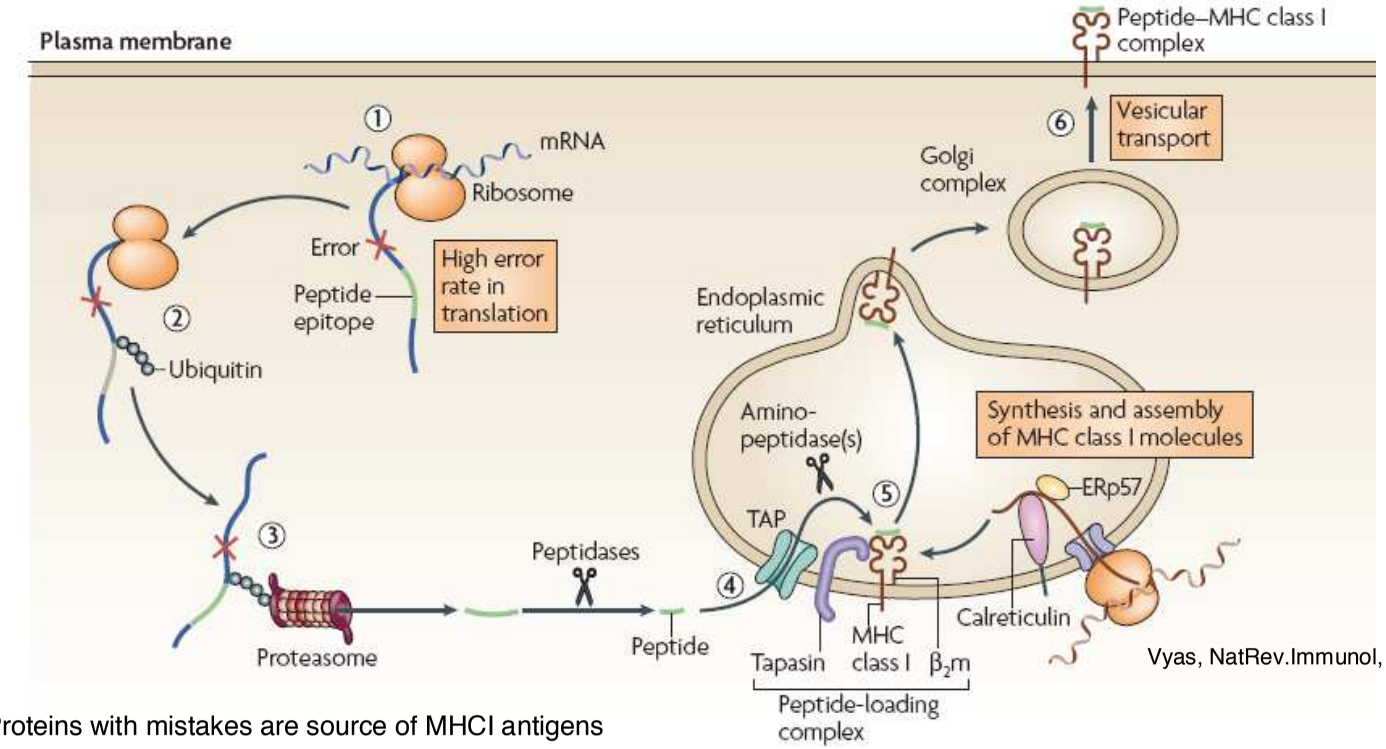
Transport of peptide MHCI complex to surface



- peptide-MHCI-complex transferred from ER via secretory pathway into Golgi network to cell surface
- post-translational modifications of MHCI (N-Glycan-Binding in ER and Golgi)
- interaction of Peptide-MHCI-complex with TCR of CD8 T cells

Abele and Tampé
FEBS Letters, 2006

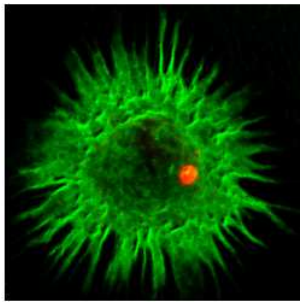
Summary: Antigen processing for MHCI



Vyas, NatRev.Immunol, 2008

1. Proteins with mistakes are source of MHCI antigens
2. Wrongly folded proteins labeled with Ub
3. Degradation of Ub-labeled proteins in peptides in proteasom, further trimming to shorter peptide by cytosolic peptidases
4. Peptides transported into ER by TAP-complex, further trimming of peptides to 8-10 aa
5. Peptides loaded onto MHC-I by peptide-loading complex (PLC) consisting of ERp57, Tapasin, and Calreticulin
6. pMHC-I complex transferred to surface via Golgi network

Dendritic cells (DCs)



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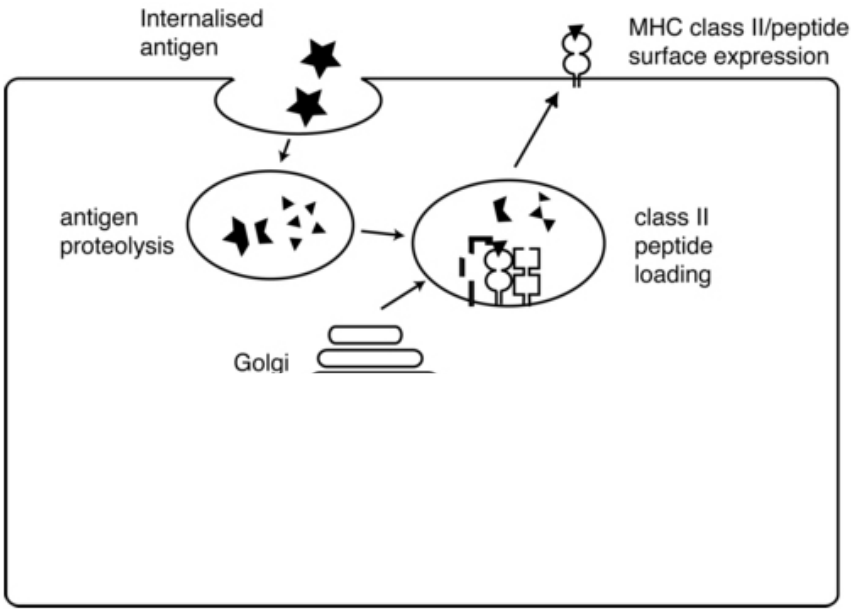
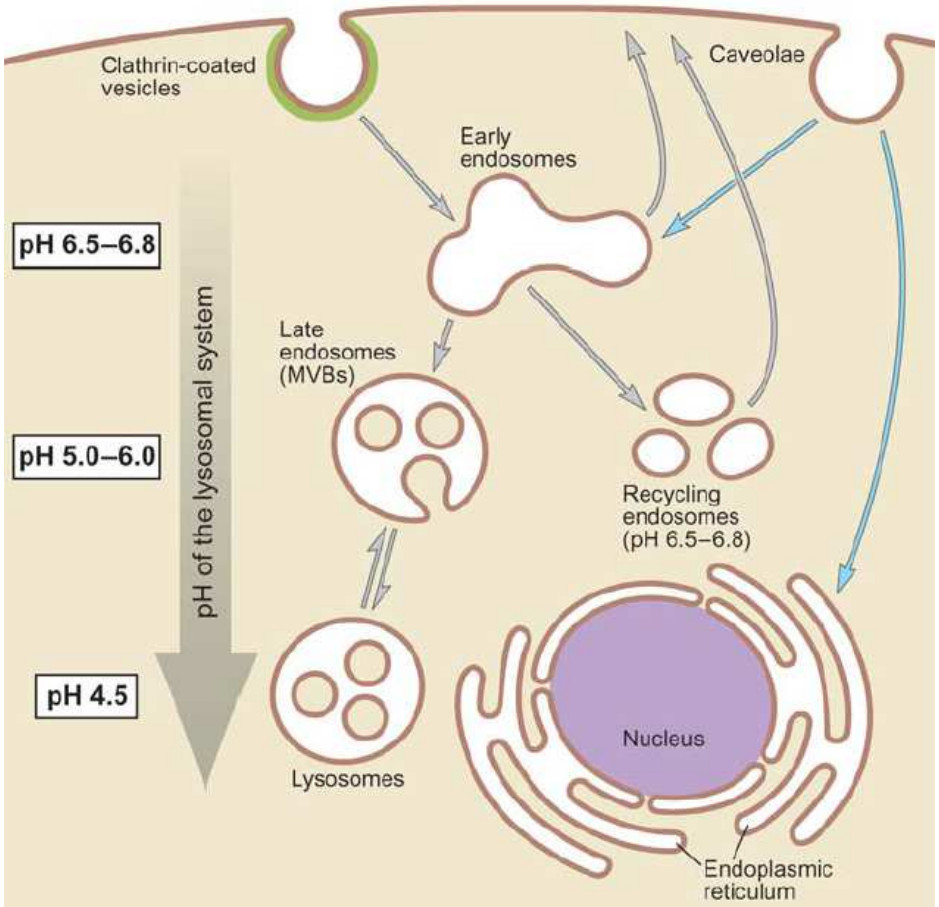
MHC class II antigen processing



- internalization of antigens (receptor-mediated endocytosis)
- proteolysis in lysosom (cleavage of thiol groups)
- transport of peptides in so called MIIC complexes

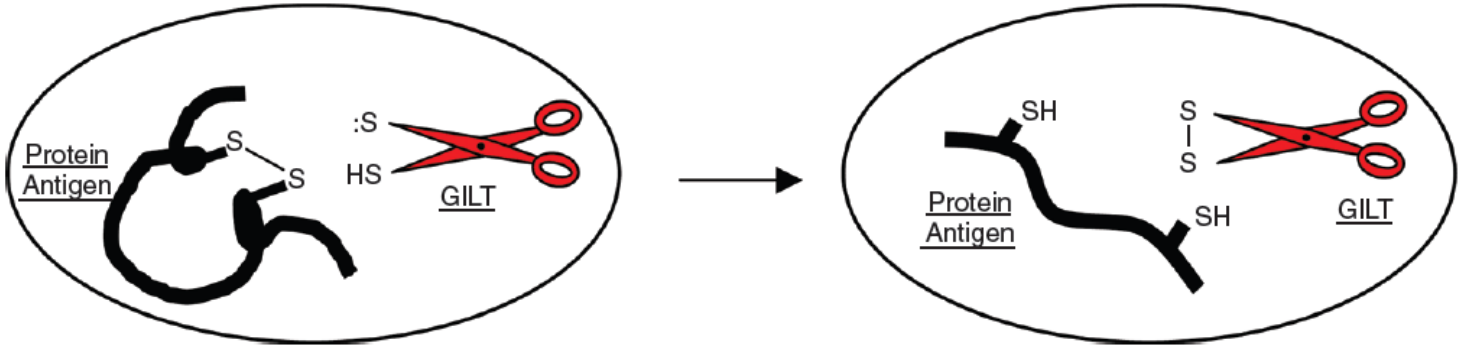
Organelles of the endocytic pathway



- receptor-recycling from 1-2 min until up to 20-30 min
- Internalization into early endosomes, late endosomes, and lysosomes
- pH drops, lysosomal hydrolase-activity increases the deeper the cargo is transported into the cell
- late endosomes and lysosomes are in exchange
- regeneration of lysosomes

Trombetta and Mellman
Annu. Rev. Immunol., 2005

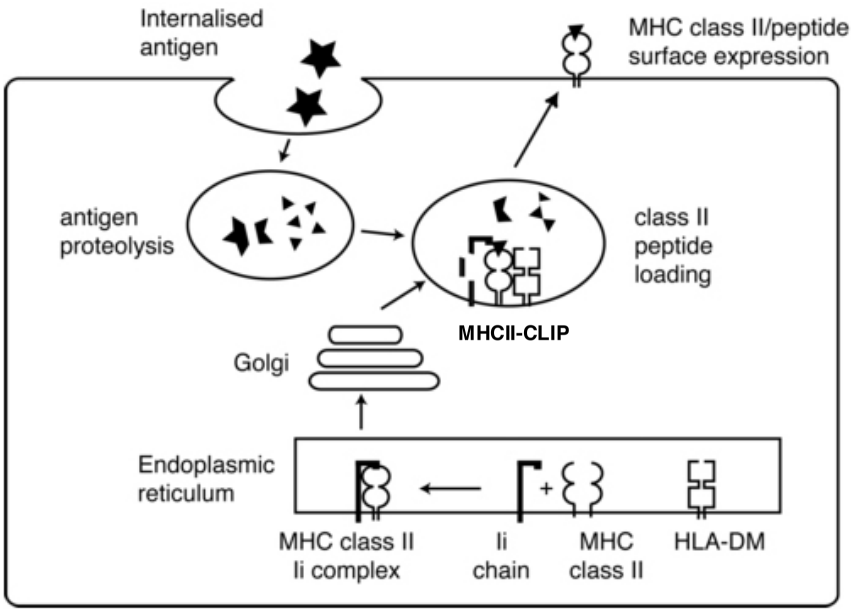
Lysosomal thiol reductase GILT



- GILT: **g**amma-**I**nterferon-inducible **l**ysosomale **T**hiol-Reductase
- active at very low pH,
- cleaves disulfide-bridges

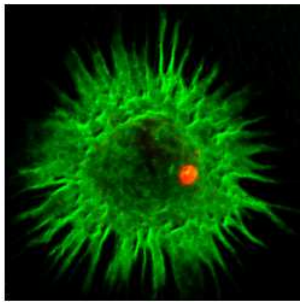
Li et al.,
Immunol. Rev. 2005

MHC class II antigen processing



- MHCII blocked by invariant chain Ii
- AEP and Cathepsines cleave invariant chain N and C terminally resulting in MHCII-Clip complex
- chaperon HLA-DM exchanges the CLIP fragment into antigenic peptides
- transport of pMHCII complex to surface
- characteristic for professional antigen presenting cells

Dendritic cells (DCs)



1. Localization
2. Antigen uptake and **antigen processing**

   (classical **/ alternative processing - cross-presentation)**
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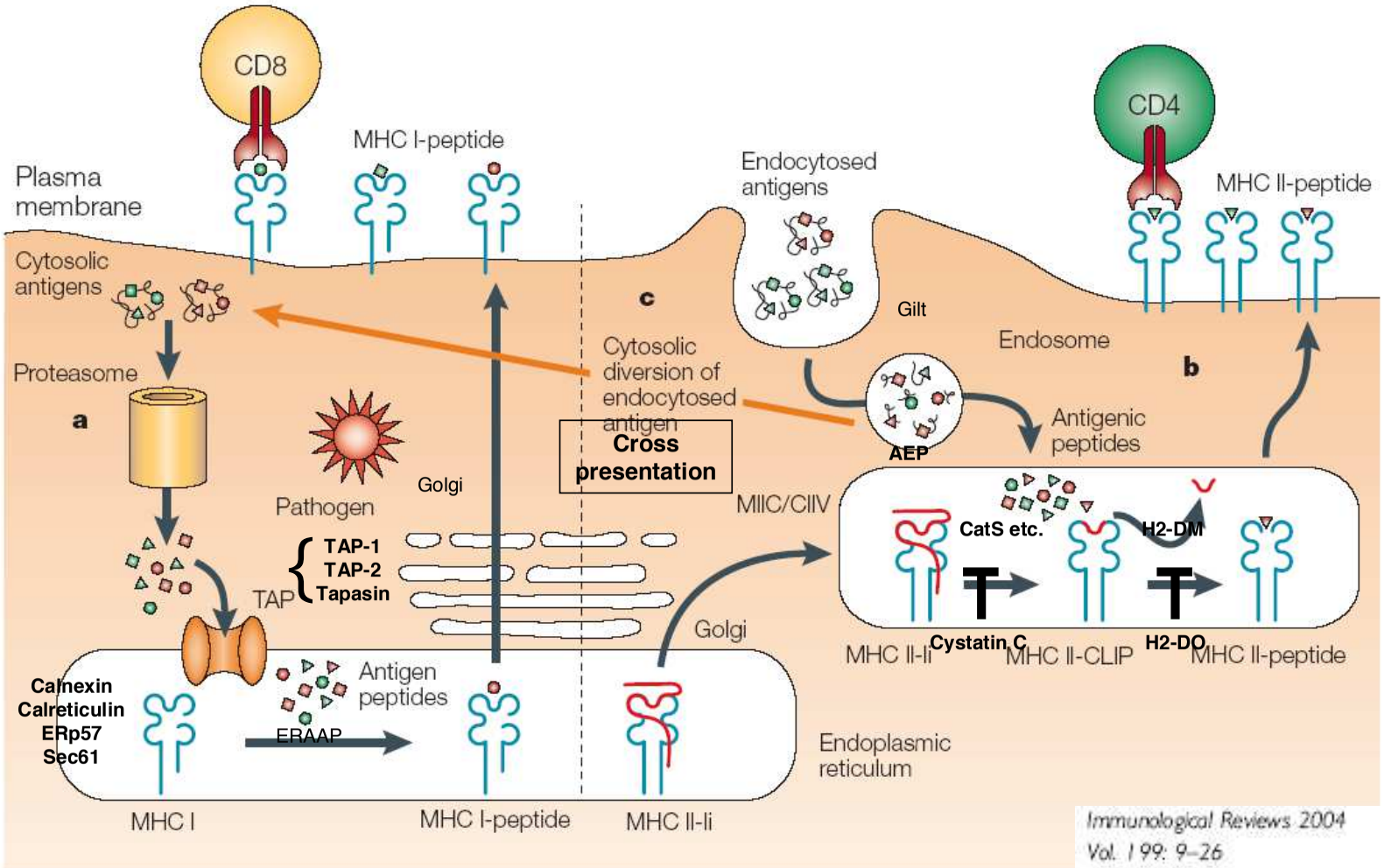
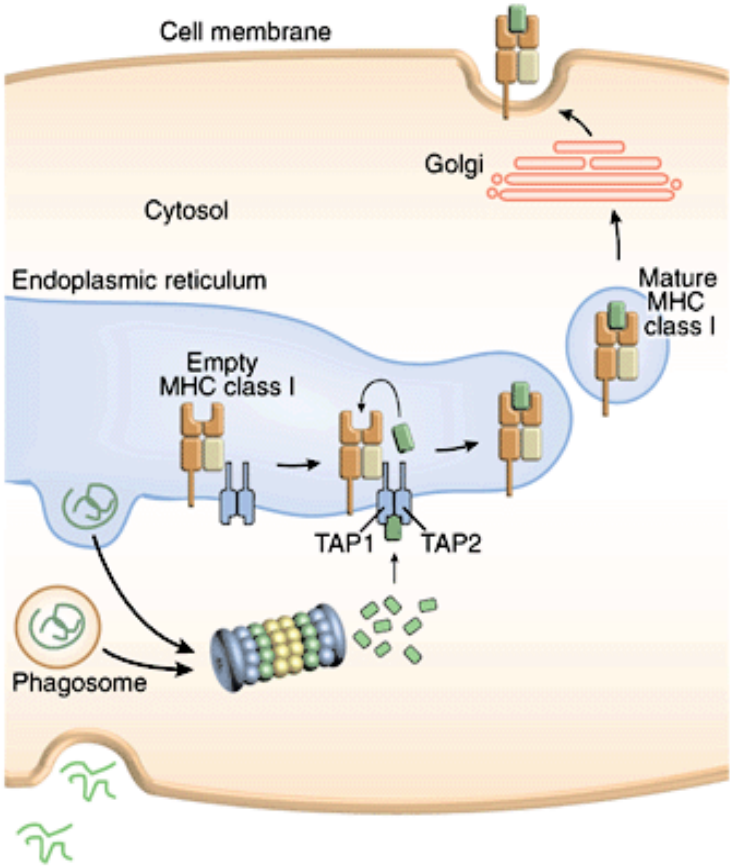
Alternative MHCI and MHCII antigen processing pathways

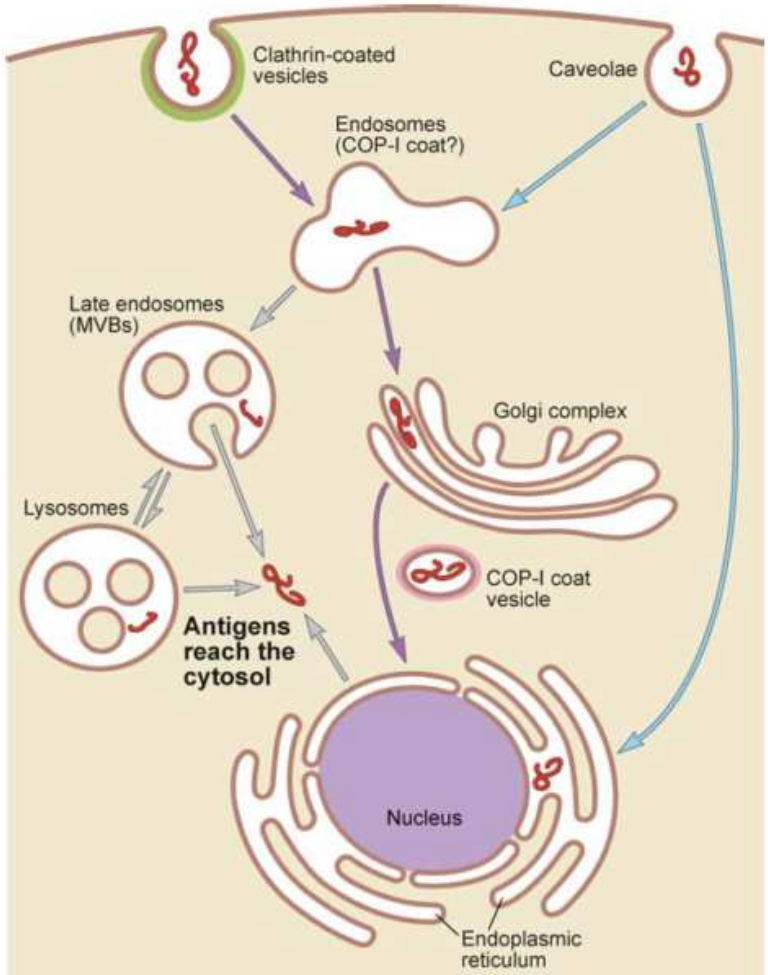


Antigen cross presentation



- antigen uptake via receptor mediated endocytosis into the so called phagosome/phagolysosome
- uptaken antigens reach the cytosol (unclear mechanism)
- degradation of self and oder viral proteins/antigens in the proteasome
- In ER: loading of peptides to MHC-I
- transport of peptid-MHC-complexes via Golgi-network to the surface

Antigen cross presentation



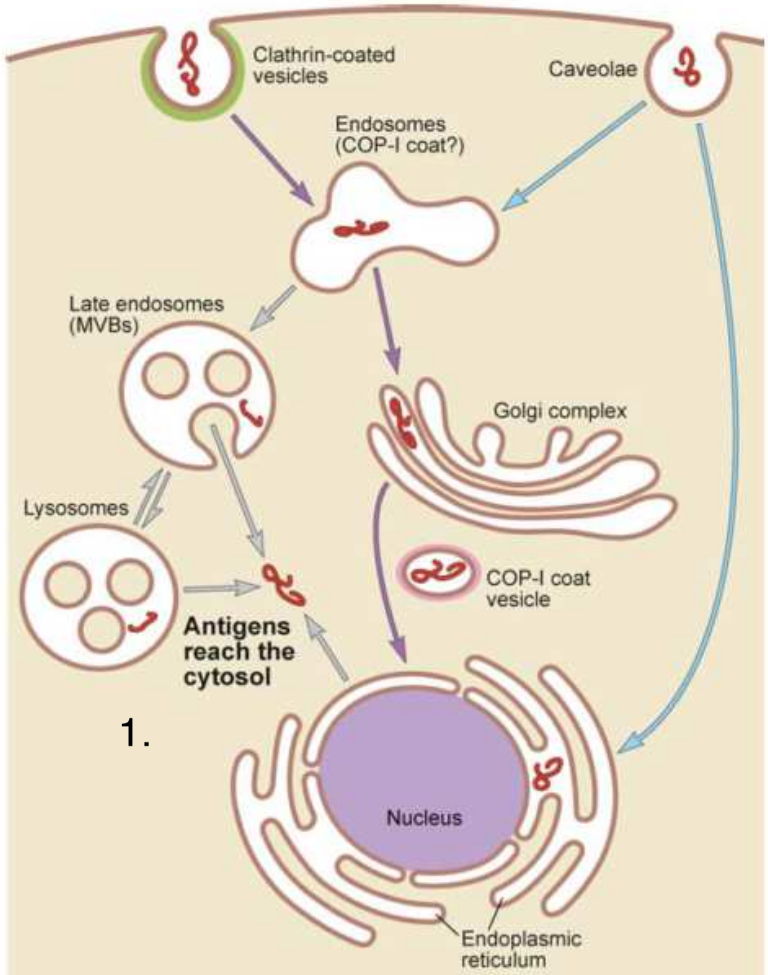
- **Cross-presentation:**

  = loading exogenous antigens onto MHC-I

  = mechanisms still unclear

Trombetta and Mellman
Annu. Rev. Immunol., 2005

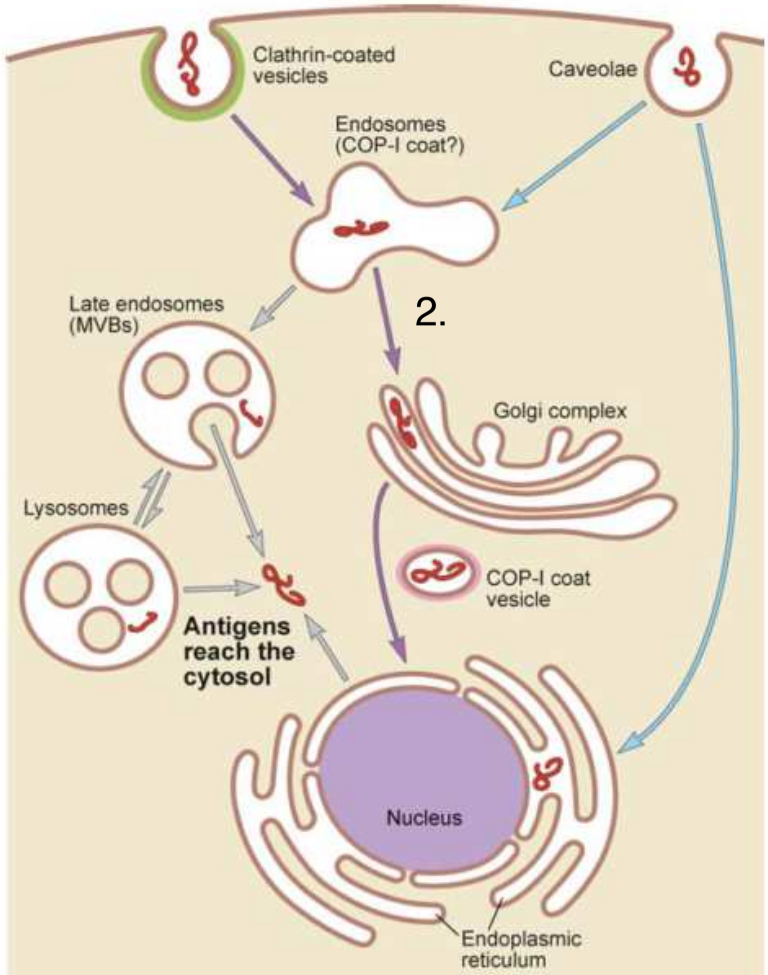
Antigen cross presentation – Hypothesis 1



- **Hypothesis 1**:
- physical disruption of endosomal/lysosomal membranes
- antigen reaches cytoplasm

Trombetta and Mellman
Annu. Rev. Immunol., 2005

Antigen cross presentation – Hypothesis 2

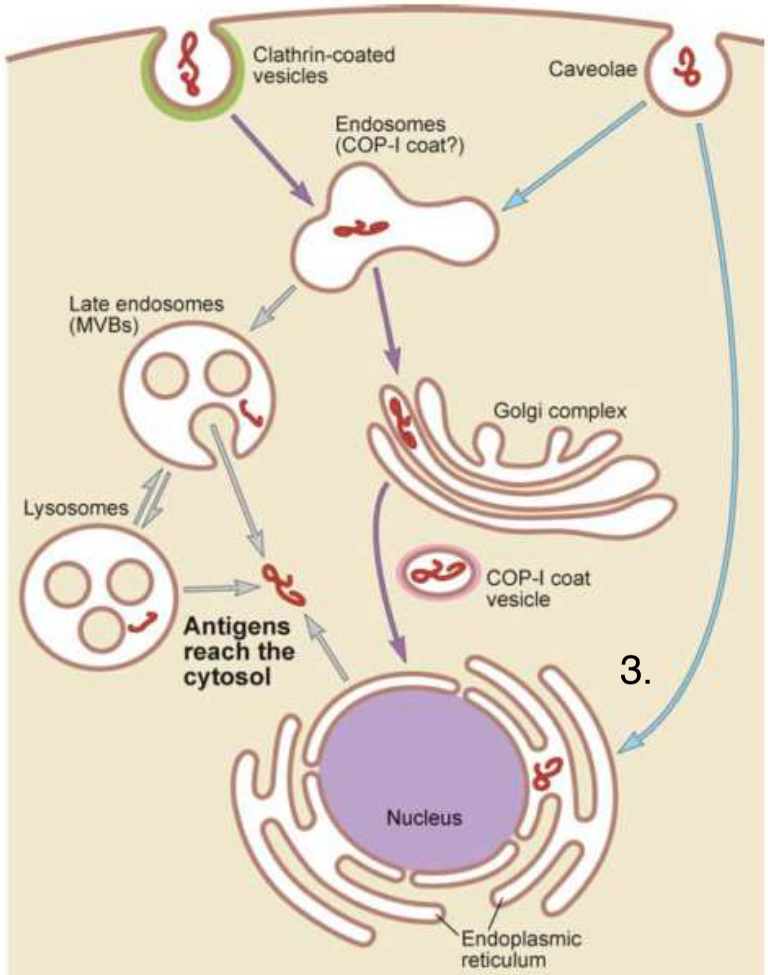


- **Hypothesis 2**:
- retrograde antigen transport

  endosome → Golgi → ER
- speculated translocation channel - transport of antigen from ER into cytoplasm

Trombetta and Mellman
Annu. Rev. Immunol., 2005

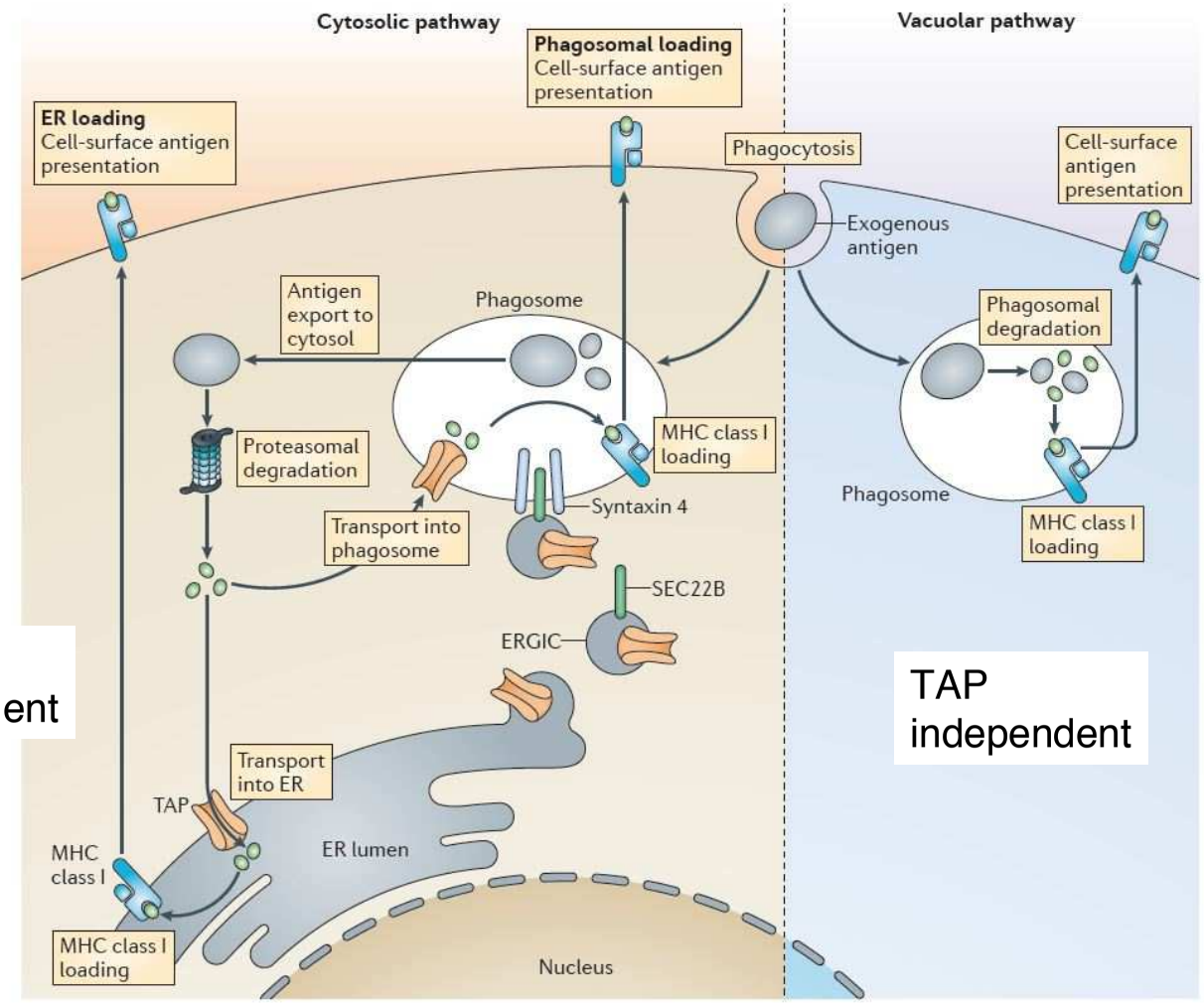
Antigen cross presentation – Hypothesis 3



- **Hypothesis 3**:
- phagosomes fuse with ER

Trombetta and Mellman
Annu. Rev. Immunol., 2005

Cytosolic and vacuolar pathway

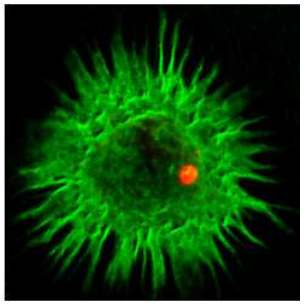


TAP dependent

TAP independent

Joffre, 2012

Dendritic cells (DCs)



1. Localization
2. Antigen uptake and **antigen processing**

   (classical MHC-I / alternative **processing - autophagy)**
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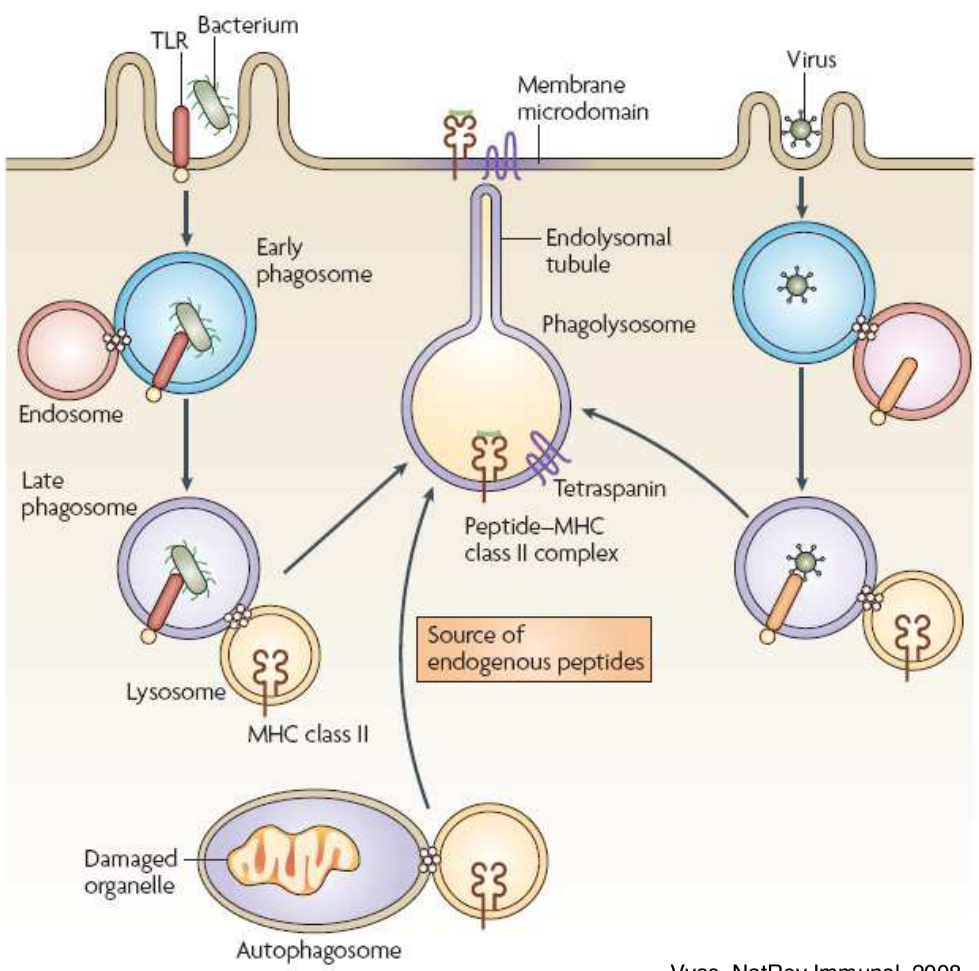
6. What makes DCs so complicated?

Yoshinori Ohsumi

Autophagy: Presentation of endogenous antigens on MHCII

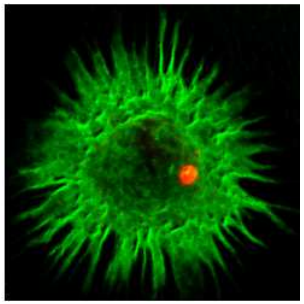


Vyas, NatRev.Immunol, 2008

- **Autophagy:**
- autophagy is a process, in which membranes assemble de novo
- presentation of endogenous antigens on MHC-Klasse-II
- pathogens, self proteins
- maturation of the phagosome by TLR activation (virus, bacteria)
- fusion of the phagosome with lysosome

  → Phagolysosome
- antigen loading onto MHC class II in the lysosome
- micro- and macroautophagy

Dendritic cells (DCs)



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Pattern recognition receptors responsible for sensing

Extracellular PRRs:

- Toll-like receptors (TLRs)
- C-Type lectin receptors (CLRs)
- Scavenger receptors

Intracellular PRRs

- NOD-like receptors (NLRs)
- RIG-I-like proteins

Pathogen-Associated Molecular Patterns

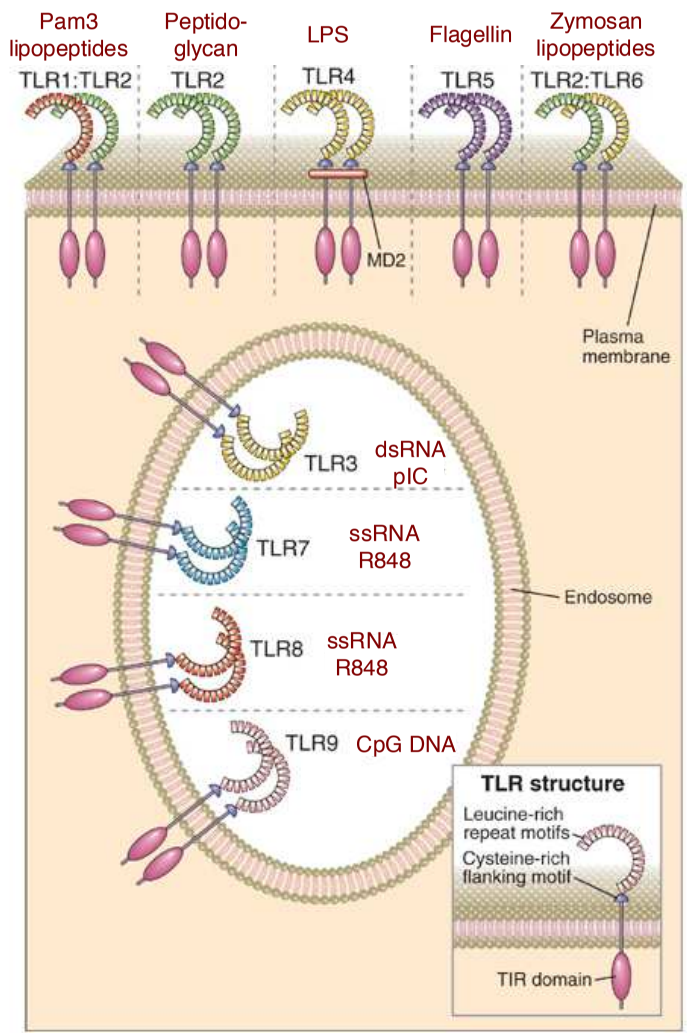
Definition:

- "Pattern Recognition Receptors" recognize structures specifically conserved in microorganisms, so-called '**Pathogen-Associated Molecular Patterns'** (PAMPs)

- Signal transduction via Pattern Recognition Receptors in antigen presenting cells leads to
  - cytokine production (proinflammatory:TNFα, IL6, IL12, anti-inflammatory: IL-10, TGFβ)
  - chemokine production (CCR7)
  - upregulation of co-stimulatory molecules (CD80, CD86)

- state of activation and antigen presentation connect the innate with the adaptive immune system

Recognition of pathogens by Toll like receptors (TLRs)



Abbas et al: Cellular and Molecular Immunology, 7e.

- recognition of structurally conserved molecules derived from microbes (PAMPs)
- signaling via MyD88 or TRIF
- activation of NFκB and IRFs
- pathogen-specific response (cytokine production, cell recruitment)

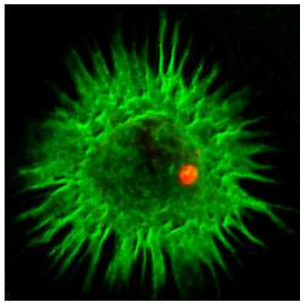
Immature and mature DCs

- **DCs in the periphery show an immature phenotype:**
  - sentinels
  - high antigen uptake and processing capacity
  - only low levels of co-stimulatory molecules
  - only low levels of MHC-I and MHC-II on the cell surface

- **Upon stimulation, DCs migrate from periphery into local lymph nodes**
  - reduced antigen uptake capacity
  - strong antigen presentation of peptide-MHC-complex
  - cytokine secretion, upregulation of co-stimulatory molecules and CCR7
  - strong interaction with T cells

- **Distinction of conventional tissue resident (semi mature) and migratory DCs, as well as plasmacytoid DCs**

Dendritic cells (DCs)



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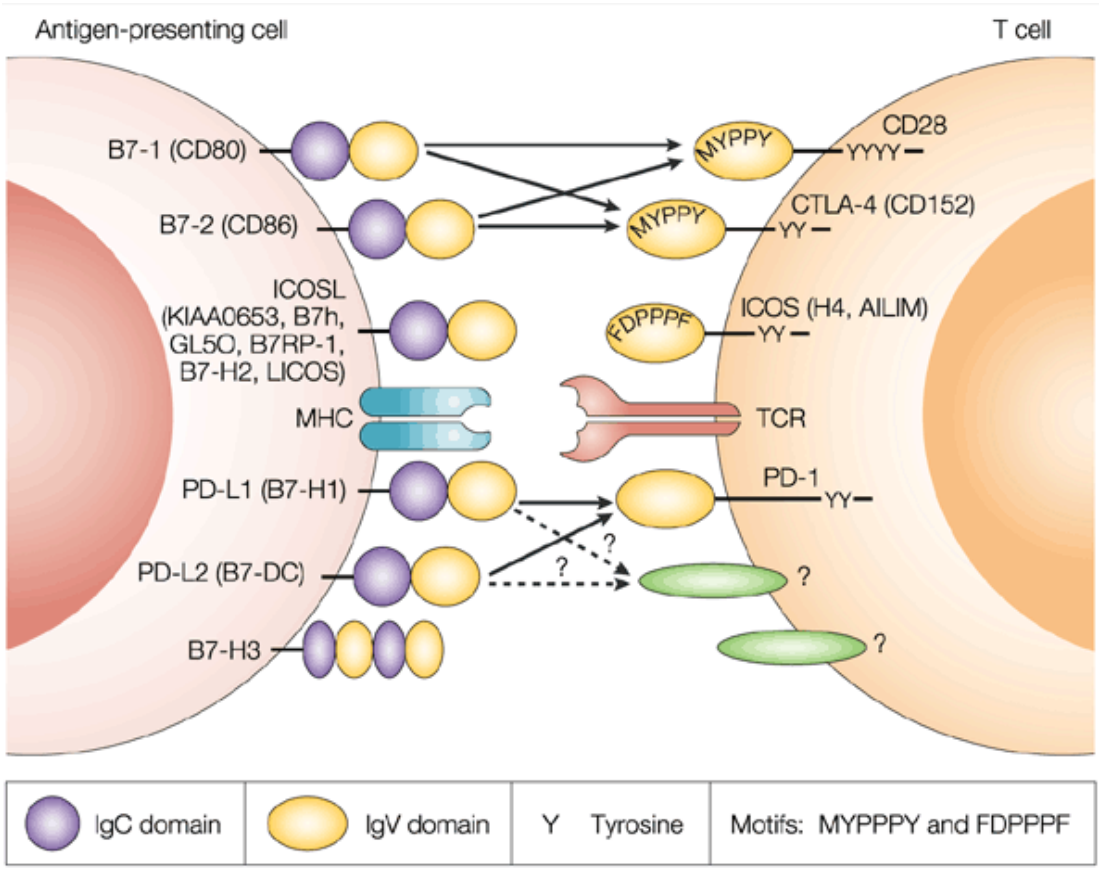
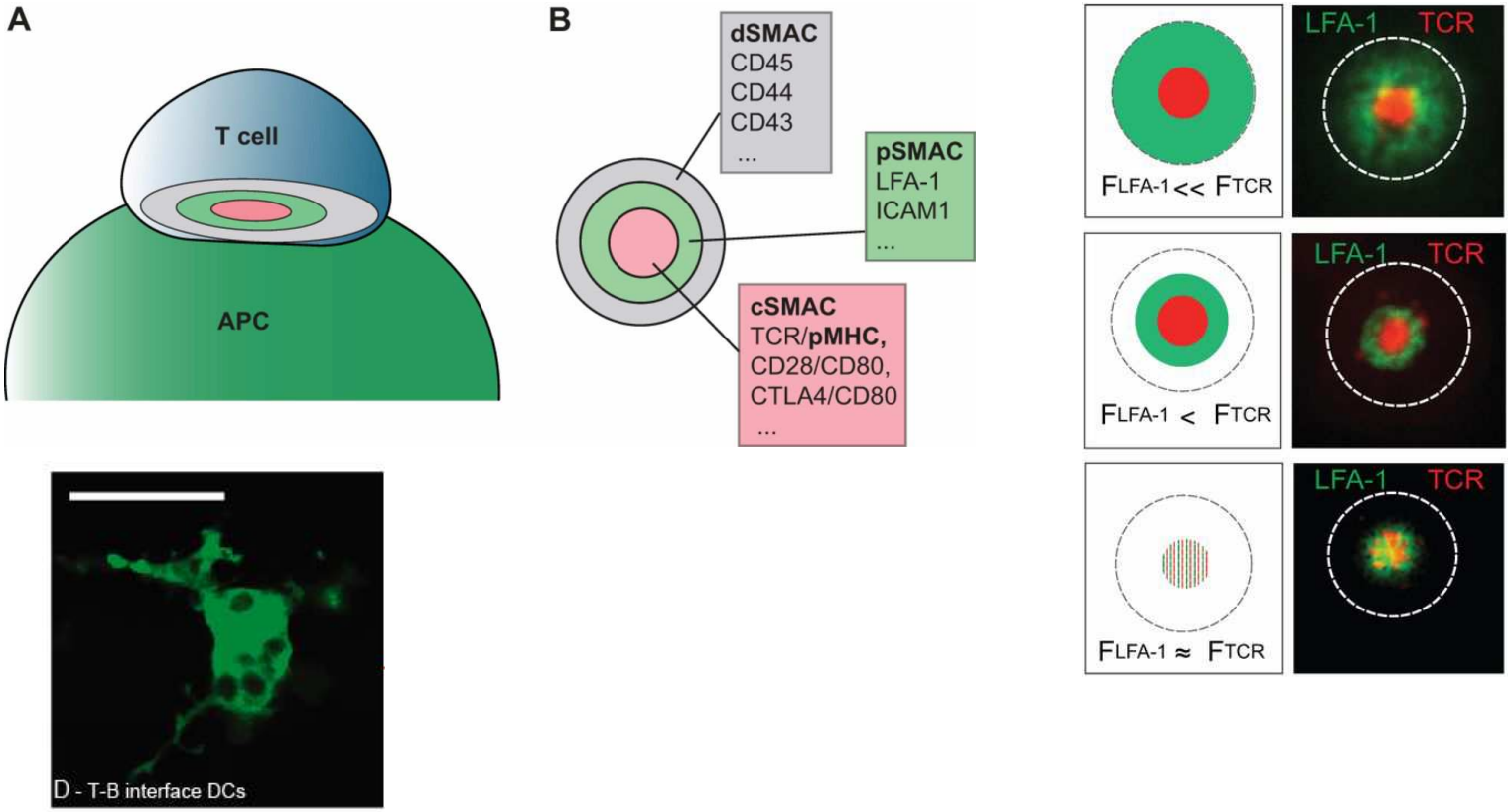
The immunological synapse



- Upregulation of co-stimulatory molecules (CD80/CD86, Ig-superfamily)

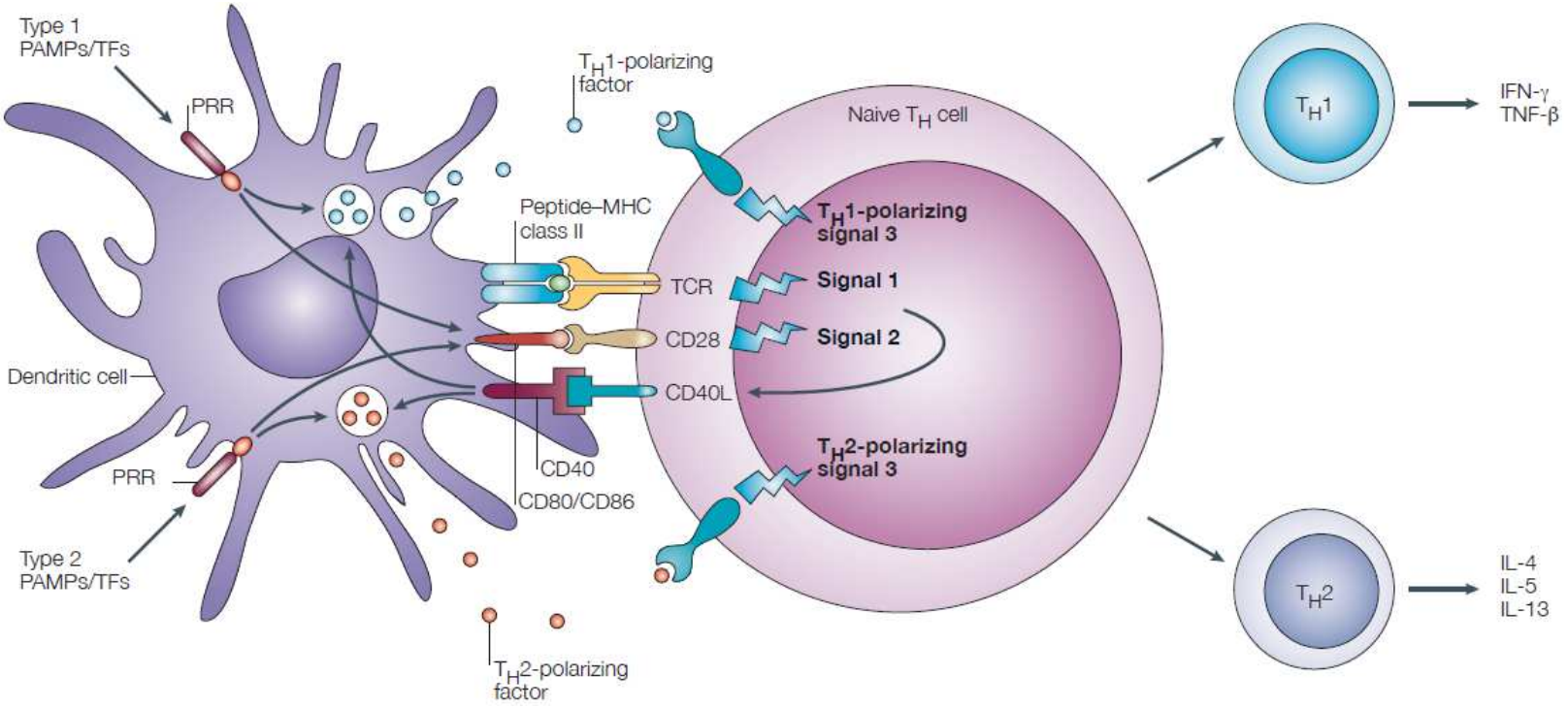
The immunological synapse



Supramolecular activation complex (SMAC)
central, peripheral, distal

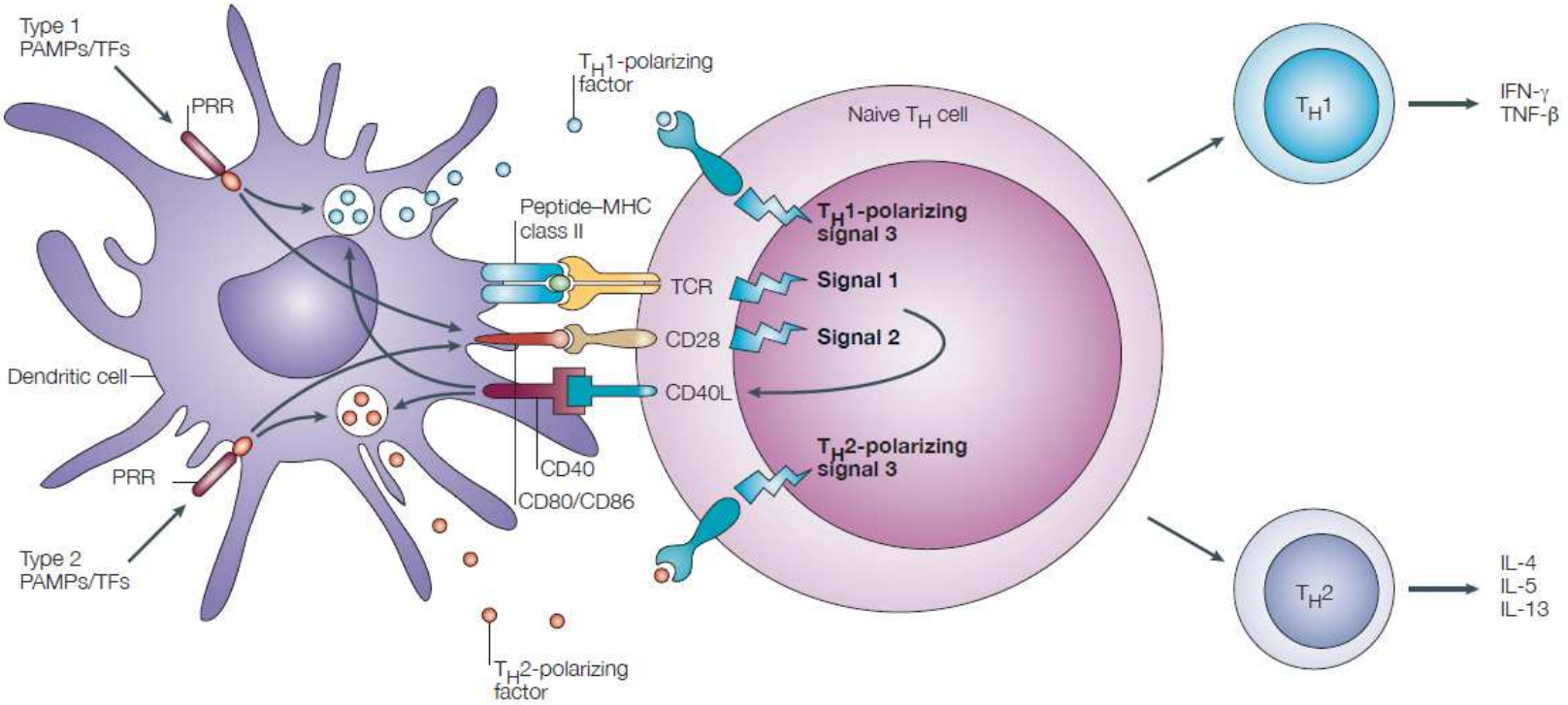
Yan Yu et al. J Cell Sci 2013;126:1049-1058

Signal 1 (antigen), Signal 2 (co-stimulation), Signal 3 (cytokines/T cell polarization)



Kapsenberg

Signal 1 (antigen), Signal 2 (co-stimulation), Signal 3 (cytokines/T cell polarization)



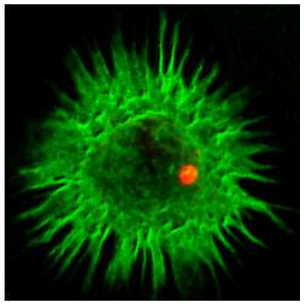
Cytokine and chemokine production strongly depend on the given stimulus

Cytokines: (secured): IL-12 necessary for T_H1 CD4 T cell polarization,

TGF-β necessary for T_{reg} polarization

Kapsenberg

Dendritic cells (DCs)



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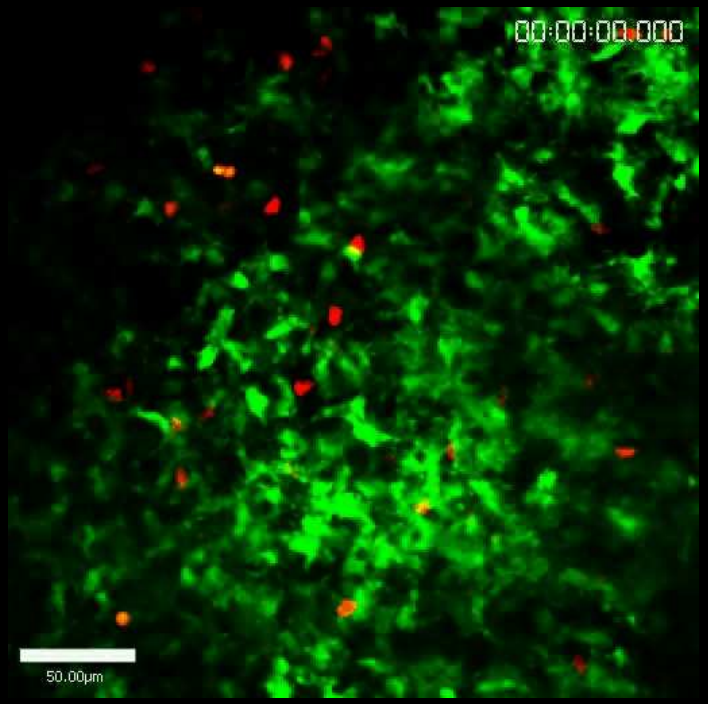
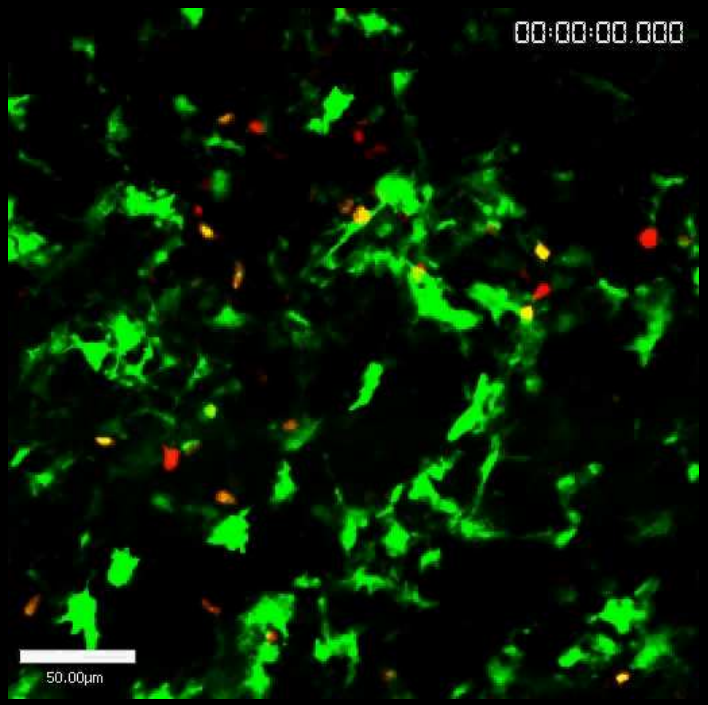
4. Antigen presentation
5. **T cell activation**
6. What makes DCs so complicated?

Antigen loaded DCs interact with antigen specific T cells independent of the DC maturation status

tolerogenic (-CD40)



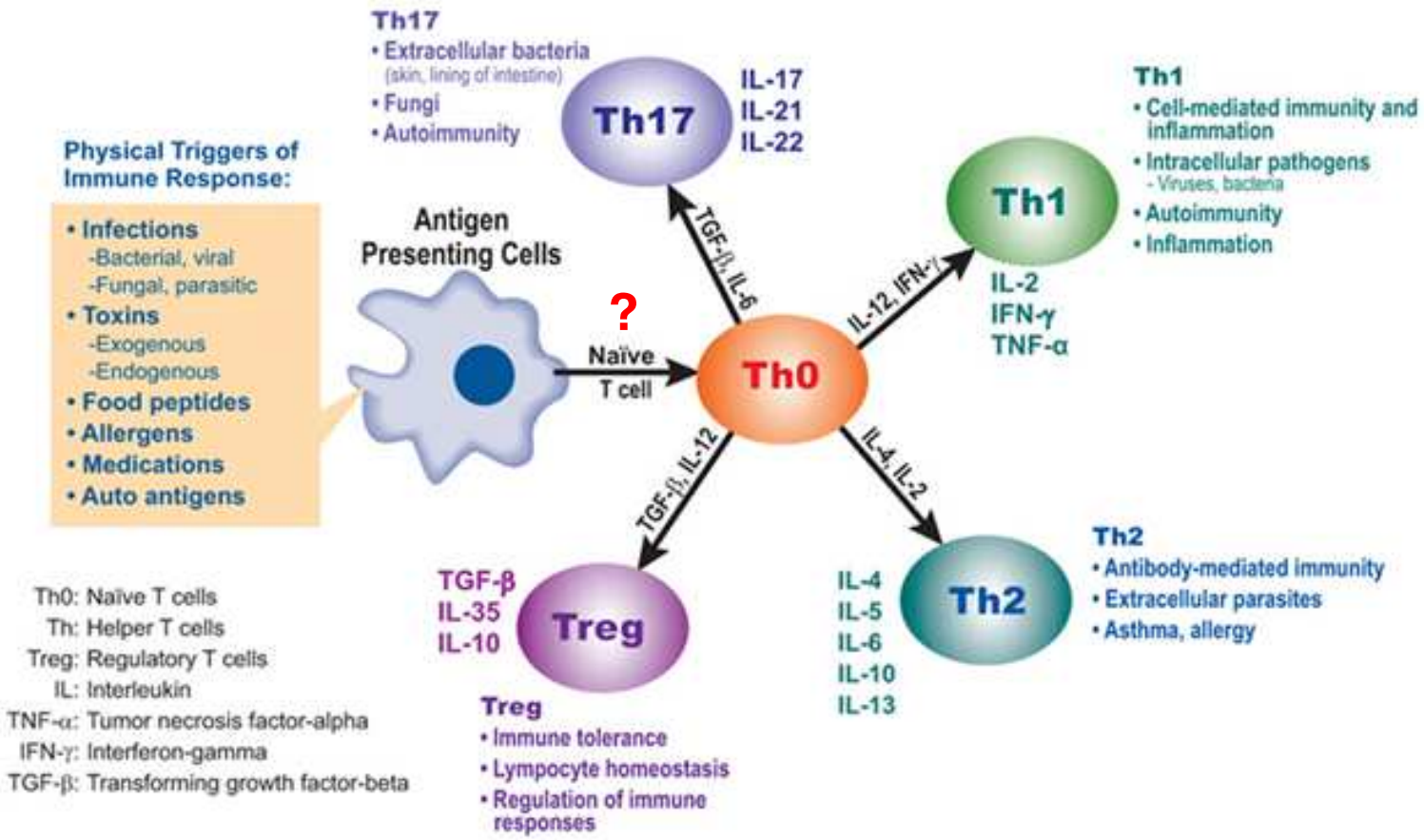
immunogenic (+CD40)



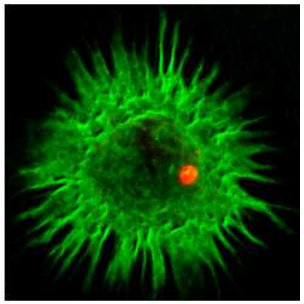
EYFP-DC
CFP-T tracks
GFP-OTII tracks

Shakar et al., Nat. Immunol. (2005)

DCs direct T cell polarization



Dendritic cells (DCs)



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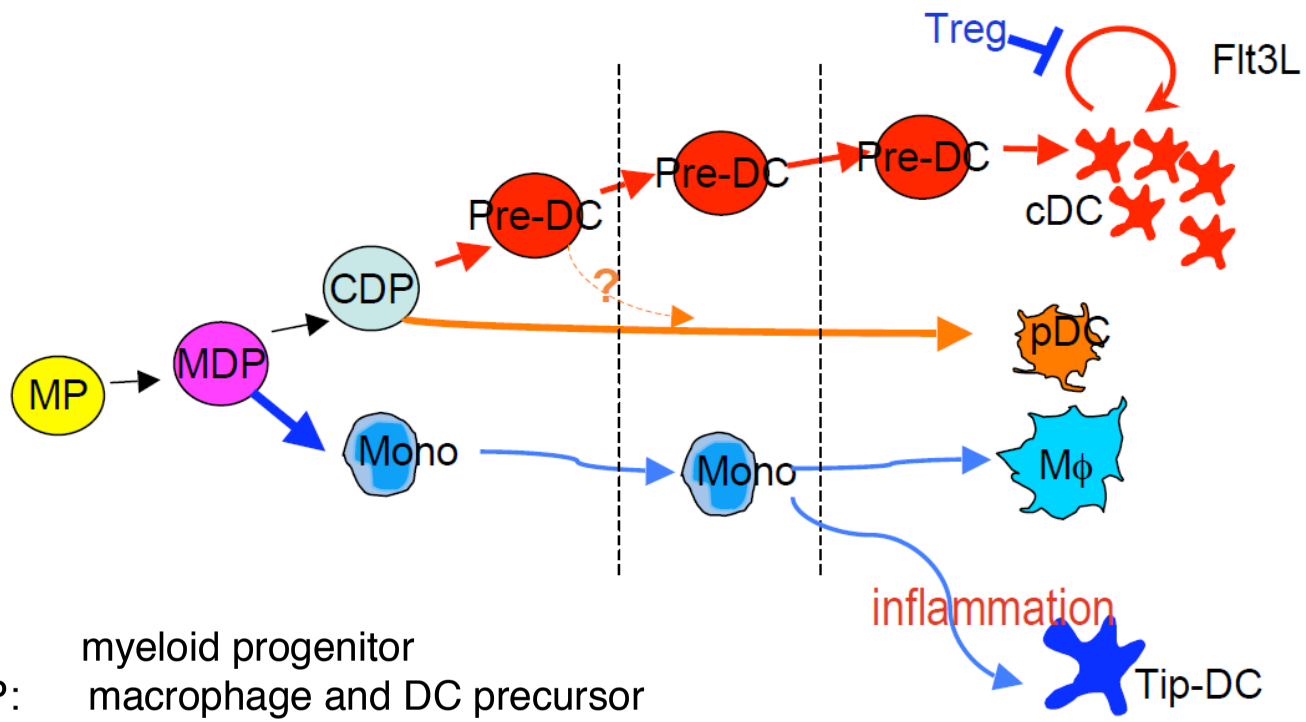
6. **What makes DCs so complicated?**
   - Very small cell numbers,
   - Comes in a variety of subsets, localized in different tissues
   - Can easily activate via preparation from tissue
   - Variety of surface markers

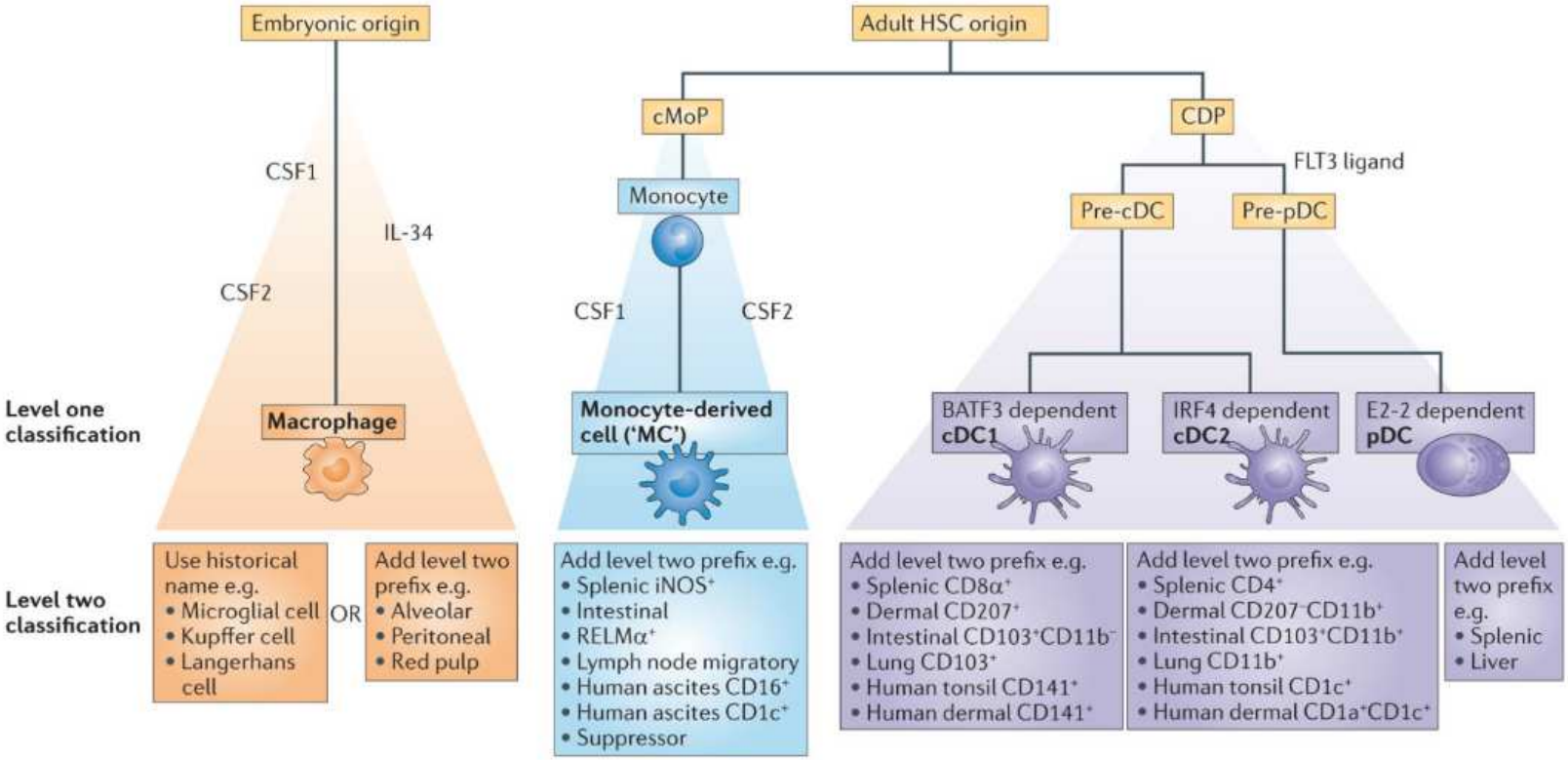
Dendritic cells (DCs)



MP: myeloid progenitor
MDP: macrophage and DC precursor
CDP: common DC precursor
Pre DC: precursors of cDCs
Tip-DCs: TNF inducible nitric oxide synthase (iNOS) -producing DCs

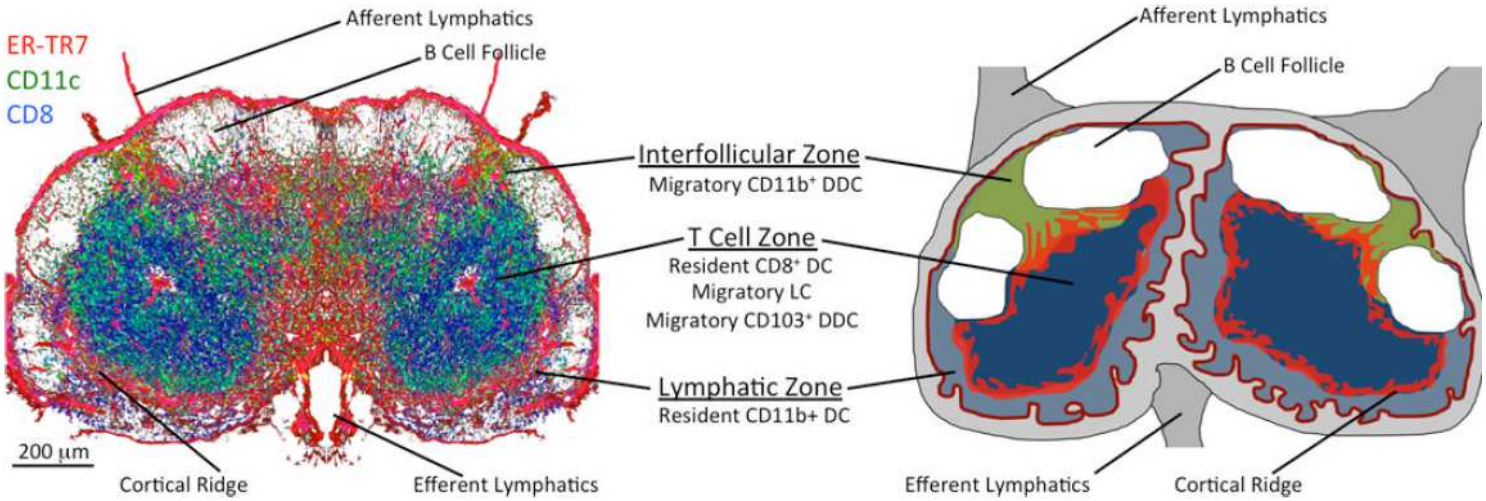
Liu K, Science. 2009 Apr 17;324(5925):392-7.

New classification of DC subpopulations



Gulliams, Nat. Rev. Immunol, 2014

Histocytometry of DC subpopulations



Gerner, Immunity, 2012

Organ distribution of murine Dendritic cells

Resident DCs

CD11c^{high} CD8^{-} CD11b^{+} ↙ ↘ CD8^{-} CD8^{-} CD4^{-} CD4^{+}	CD11c^{high} CD8^{+} CD4^{-} CD11b-	CD11c^{low} B220^{+} GR1^{+} PDCA-1^{+} SiglecH^{+}	CD11c+ CD8^{int} Langerin^{+} CD103^{+}	CD11c^{+} Langerin^{+} Epcam^{+} CD103^{-} CD11b^{+}	CD11c^{+} Langerin^{+} CD103^{+}	CD11c^{+} Langerin^{+} CD103^{-}	CD11c^{+} Langerin^{-} CD11b^{-}	CD11c^{+} Langerin^{-} CD11b^{High}
(myeloid)	('lymphoid')	plasmacytoid	Migratory LC	Langerhans' cells	CD207^{+} DDC	CD207^{+} mLCs	CD207^{-} DDC	
Spleen Lymph node Peyer's Patch Thymus Liver			Skin draining LN	Epidermis	Dermis			

Further distinction of DC subpopulations in lamina propria, lung, thymus…

Murine resident CD8$^+$ DCs

CD11c^{high}
CD8+/CD11b-

(‘lymphoid’)

- **cross-presentation of endocytosed antigens on MHCI**
- activation of CD8$^+$ cytotoxic T cells (leading to IFN-γ, Granzyme)
- also able to activate T_H1 CD4$^+$ helper T cells (IFN-γ), by antigen presentation on MHCII
- uptake of tumor material, virus-infected cells
- main producers of IL-12
- additional receptors: DEC205, XCR-1, Clec9A
- BATF3 dependent

- Cross-presentation demonstrated also for some CD103$^+$ migratory DCs

Murine resident CD8$^-$ DCs

CD11c^{high}
CD8-/CD11b+

(‘myeloid’)

- important for anti-bacterial, anti-parasite immune response
- uptake of ‘Blood-born Antigens’
- antigen presentation on MHC-class II
- activation of TH2 CD4$^+$ T-helper cells (IL-4)
- upregulation and secretion of Notch-ligands
- further receptors: DCIR2, SIRP α
- IRF4 dependent

Murine plasmacytoid DCs

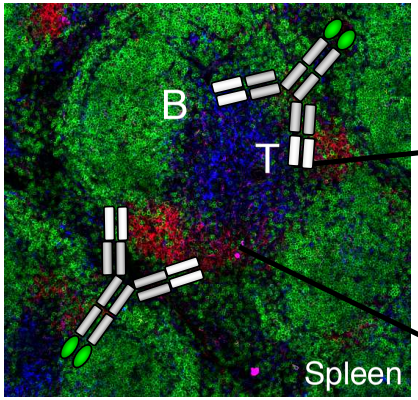
CD11c^{low}
PDCA-1^{+}

plasmacytoid

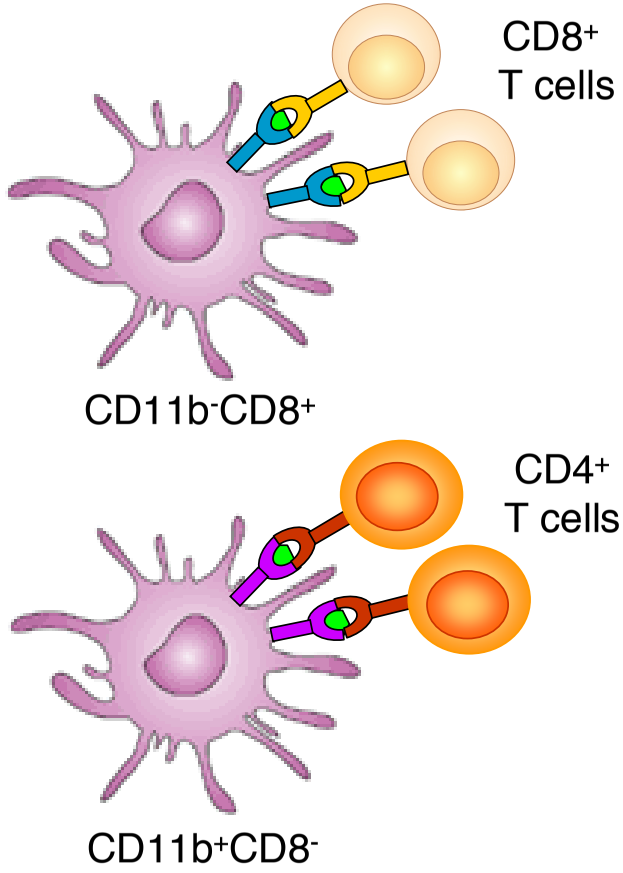
- morphological similar to antibody producing plasma cells (B cells)
- production of high amounts of Interferon I (IFN-α, IFN-β) upon virus infection
- maintenance of peripheral tolerance
- further receptors: PDCA-1, SiglecH, partly CCR9
- E2-2 dependent

In vivo antigen targeting of DC subpopulations induces different T cell responses in mice

αDEC205-Ova



33D1/αDCIR2-Ova



Heidkamp et al., Science Immunology, in press
Lehmann et al., Vaccines, 2016
Heidkamp et al., Encycl. of Cell Biology, 2016
Milo et al., Blood, 2013
Lindmark et al., J Autoimm, 2013
Loschko et al., JI, 2011-2
Loschko et al., JI, 2011-1
O'Kamphorst et al., JI, 2010
Do et al., EJI, 2010
Yamazaki and Dudziak, JI, 2008
Dudziak et al., Science, 2007
Soares et al., JEM, 2007
Shakhar et al., Nat. Immunol. 2005
Lindquist et al., Nat. Immunol., 2004

Thank you for your attention !