Graduate Program in Immunology

BMI5905 - Effector Mechanisms of Immune Response

# Antigen Presentation and Peripheral Tolerance

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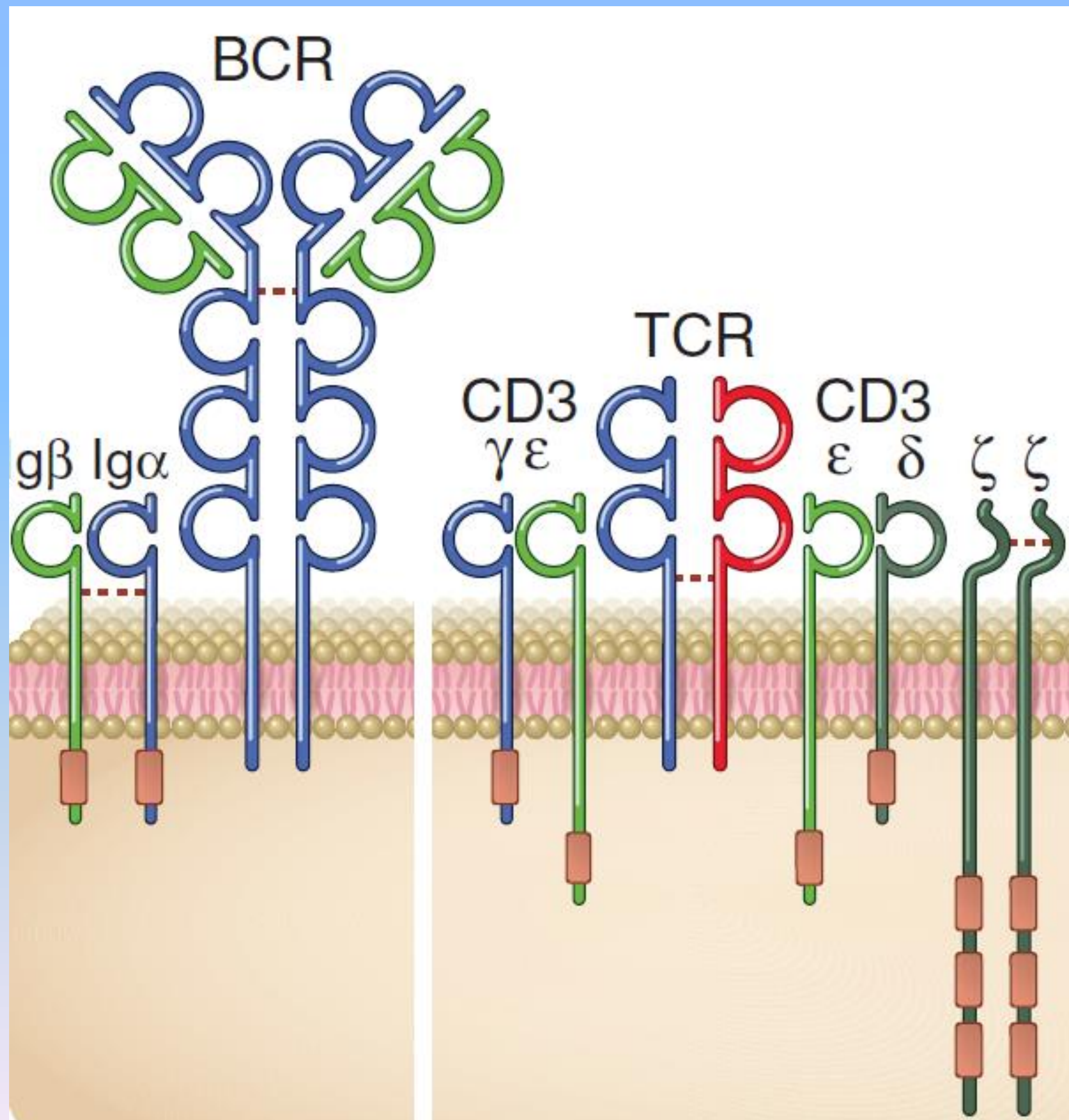
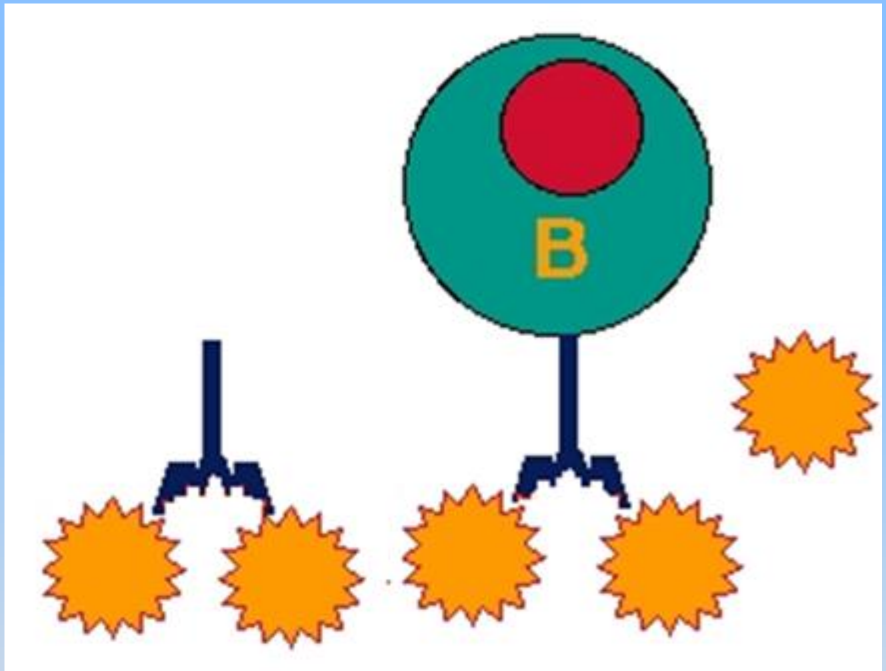
Department of Immunology
Institute of Biomedical Sciences
University of Sao Paulo

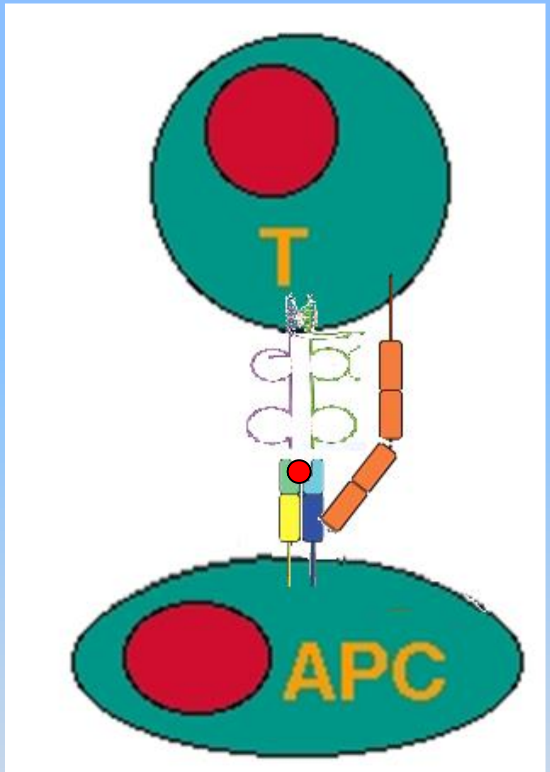
# Antigen Receptors of Acquired Immunity




*Abbas, Lichtman, Pillai, 10th. Edition, 2021.*

# Antigen Recognition by Lymphocyte Receptors

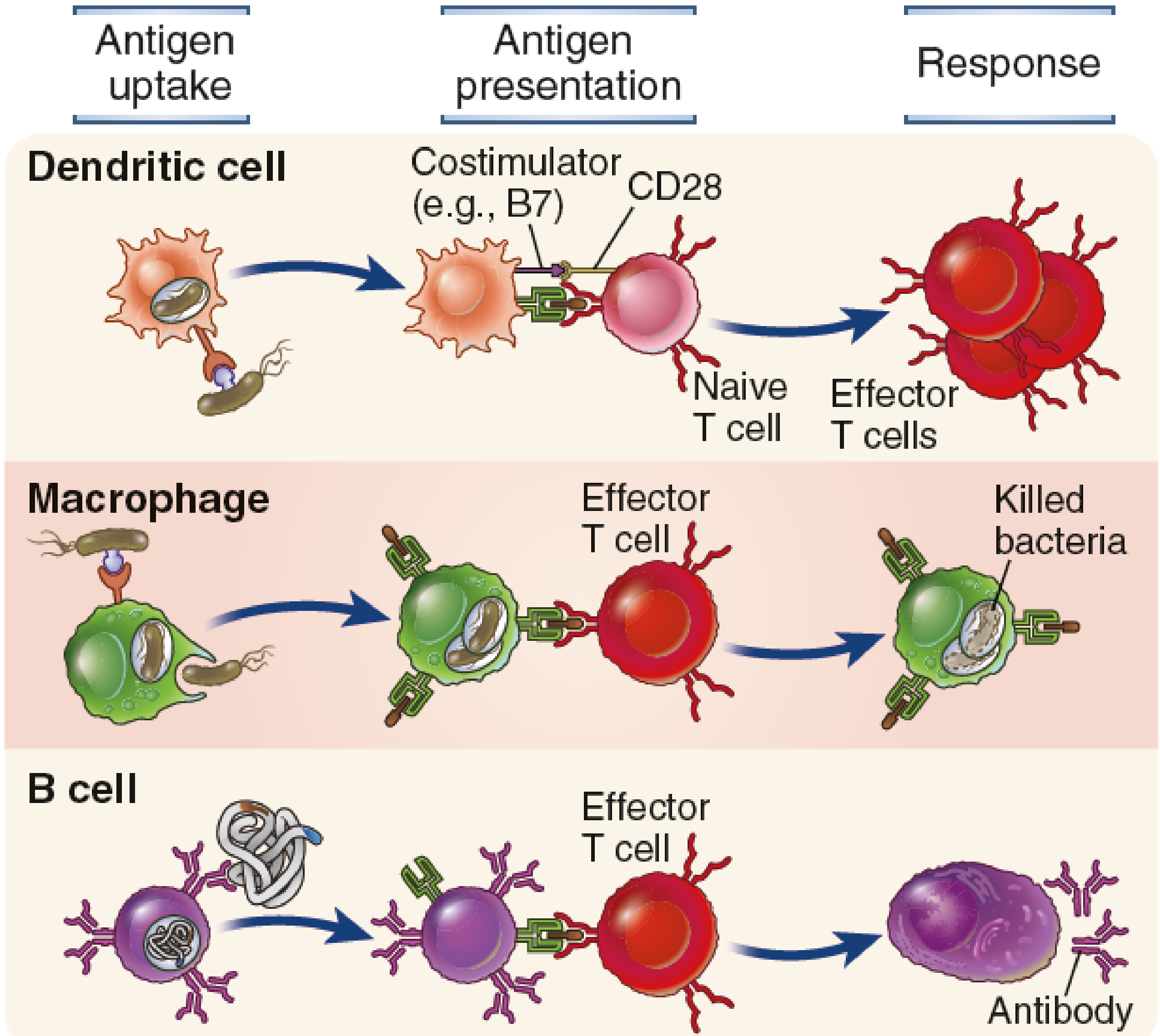



*BCRs recognize antigens on both native or denaturated conformation*

*TCRs do not recognize antigens directly. Small parts of protein antigens (peptides) need to be bound to MHC molecules and displayed by antigen presenting cells (APC)*

# Antigen Presenting Cells



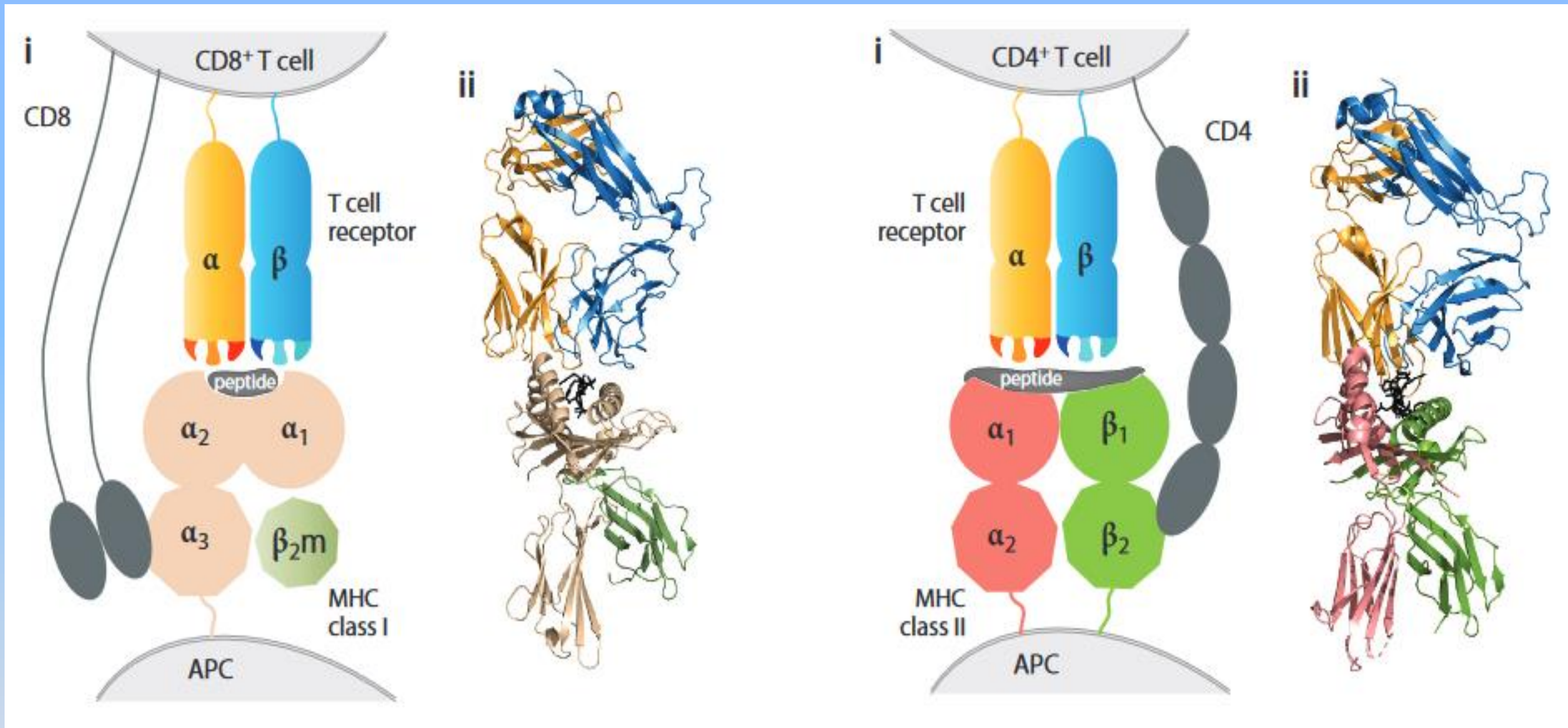

**Naive T cell activation: clonal expansion and differentiation into effector T cells**

**Effector T cell activation: activation of macrophages (cell-mediated immunity)**

**Effector T cell activation: B cell activation and antibody production (humoral immunity)**

*Abbas, Lichtman, Pillai, 10th. Edition, 2021.*

# MHC-peptide/TCR interactions




**HLA-A**
**HLA-B**
**HLA-C**

**HLA-DP**
**HLA-DQ**
**HLA-DR**

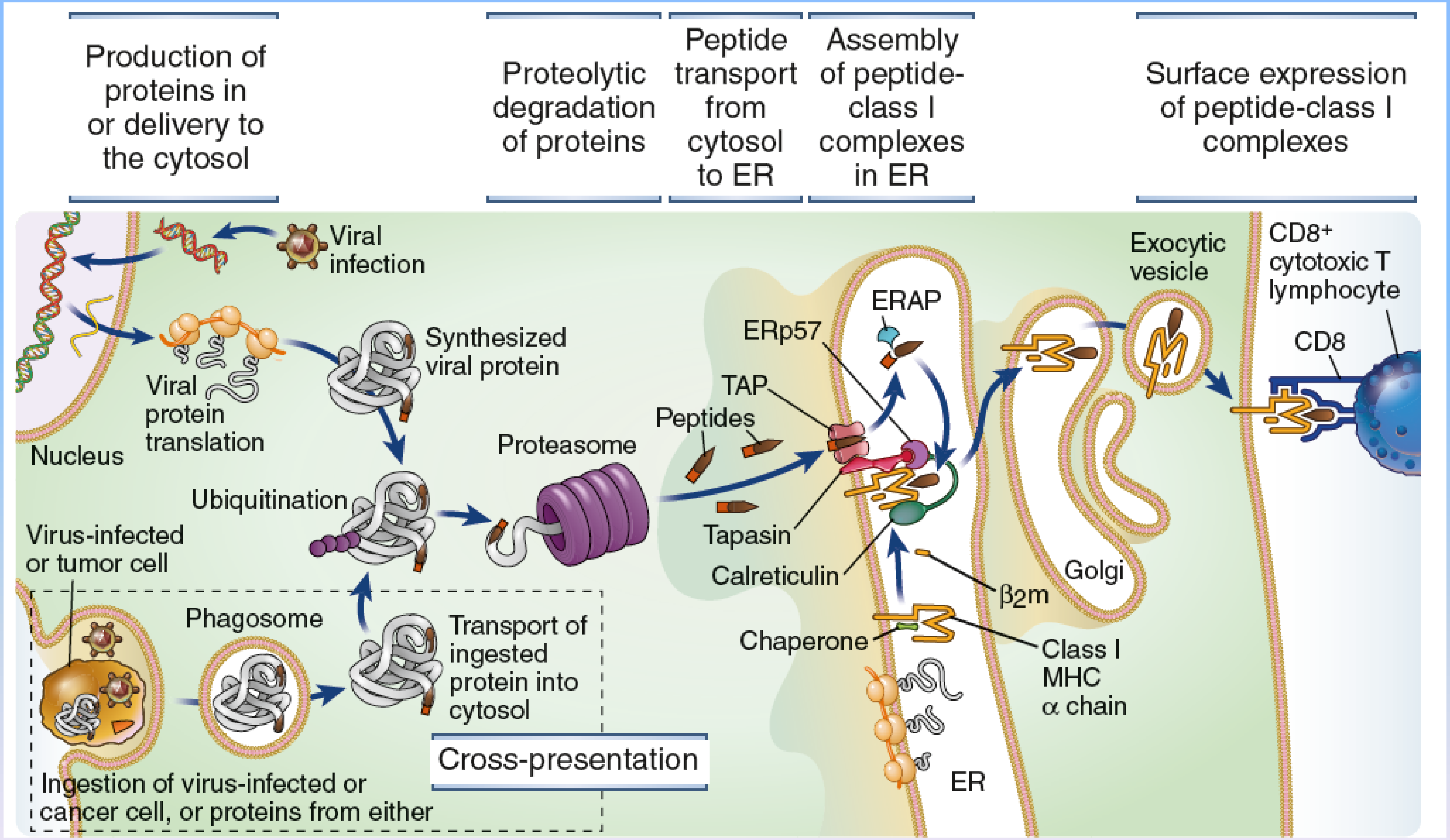
*Djaoud & Parham, Annu. Rev. Biochem., 20;89:717-739, 2020.*

# Peptide binding to MHC molecules and allotypes


| HLA allotype | Number of HLA allotypes |
|---|---|
| HLA-A | 3,629 |
| HLA-B | 4,572 |
| HLA-C | 3,447 |
| HLA-E | 12 |
| HLA-F | 6 |
| HLA-G | 19 |
| HLA class I | 11,685 |
| HLA-DP | 3,160 |
| HLA-DQ | 1,278 |
| HLA-DR | 1,055 |
| HLA-DM | 11 |
| HLA-DO | 8 |
| HLA class II | 5,512 |

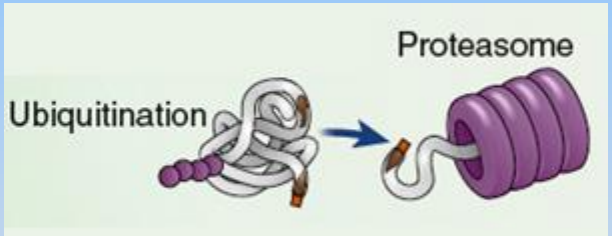
*Djaoud & Parham, Annu. Rev. Biochem., 20;89:717-739, 2020.*

# MHC Class I Pathway




*Abbas, Lichtman, Pillai, 10th. Edition, 2021.*

# Ubiquitin/Proteasome System



# Ubiquitin

- *Identified in 1974, sequenced one year later*
- *Present in all living organisms*
- *76 aminoacids (8.5 kDa)*

*-Extremely conserved:*

*Human X Yeast = 96% identity*

BIOCHEMISTRY, VOL. 14, NO. 10, 2214-2218, 1975

**The Complete Amino Acid Sequence of Ubiquitin, an Adenylate Cyclase Stimulating Polypeptide Probably Universal in Living Cells†**

David H. Schlesinger,* Gideon Goldstein, and Hugh D. Niall‡

*MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG*

*Schlesinger et al., Biochemistry, 14(10): 2214-8, 1975.*

# Ubiquitin

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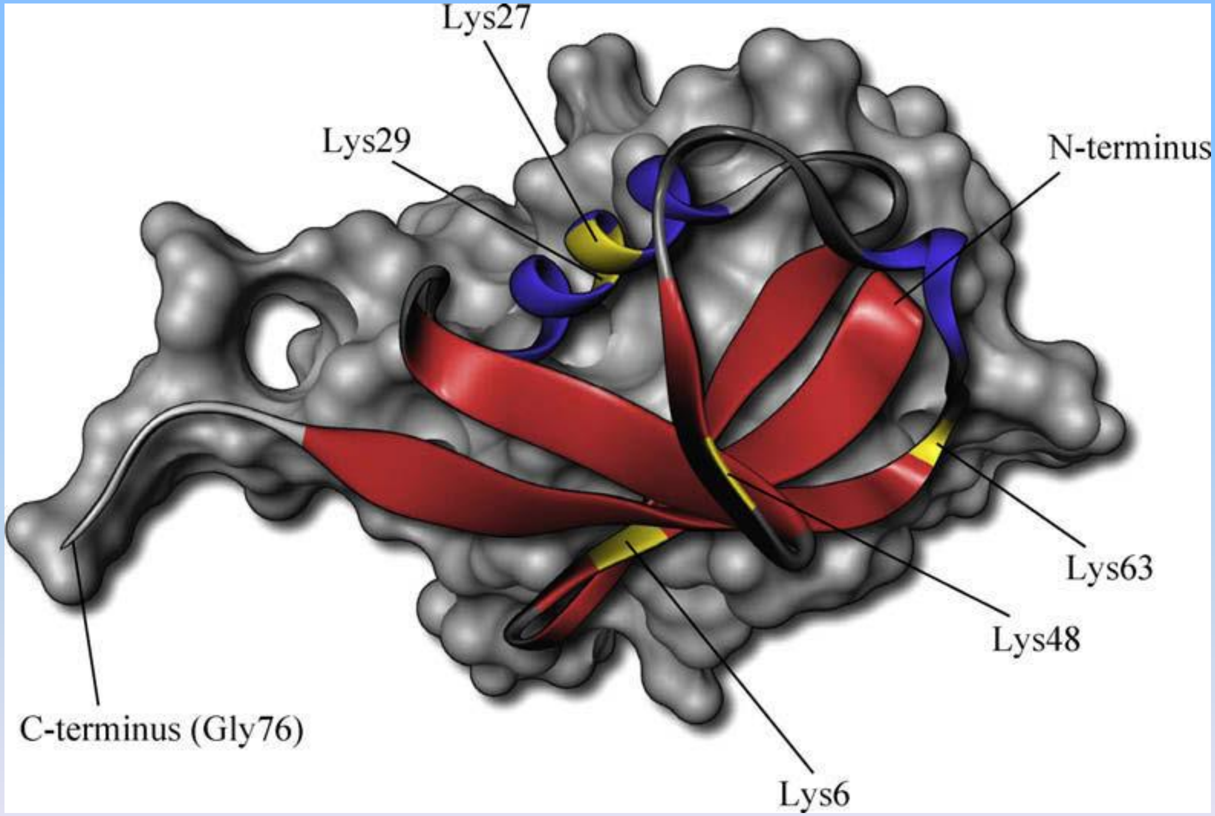



MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG

Jung et al., Molecular Aspects of Medicine, 30: 191-296, 2009.

# Elucidation of basic functions of ubiquitin and protein ubiquitination pathways

## The Nobel Prize in Chemistry 2004
Aaron Ciechanover, Avram Hershko, Irwin Rose

The Nobel Prize in Chemistry 2004 was awarded jointly to Aaron Ciechanover, Avram Hershko and Irwin Rose *"for the discovery of ubiquitin-mediated protein degradation"*.

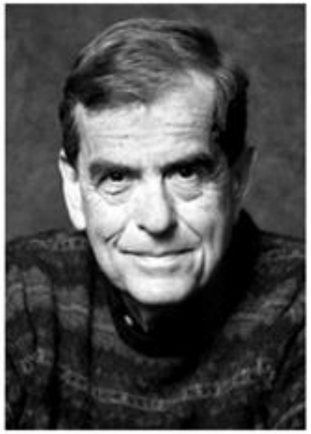


Photo: D. Porges

### Aaron Ciechanover

**Aaron Ciechanover**

**Born:** 1 October 1947, Haifa, British Protectorate of Palestine (now Israel)

**Affiliation at the time of the award:** Technion – Israel Institute of Technology, Haifa, Israel

**Prize motivation:** "for the discovery of ubiquitin-mediated protein degradation"

**Field:** Biochemistry


Photo: D. Porges

### Avram Hershko

**Avram Hershko**

**Born:** 31 December 1937, Karcag, Hungary

**Affiliation at the time of the award:** Technion – Israel Institute of Technology, Haifa, Israel

**Prize motivation:** "for the discovery of ubiquitin-mediated protein degradation"

**Field:** Biochemistry

### Irwin Rose

**Irwin Rose**

**Born:** 16 July 1926, Brooklyn, NY, USA

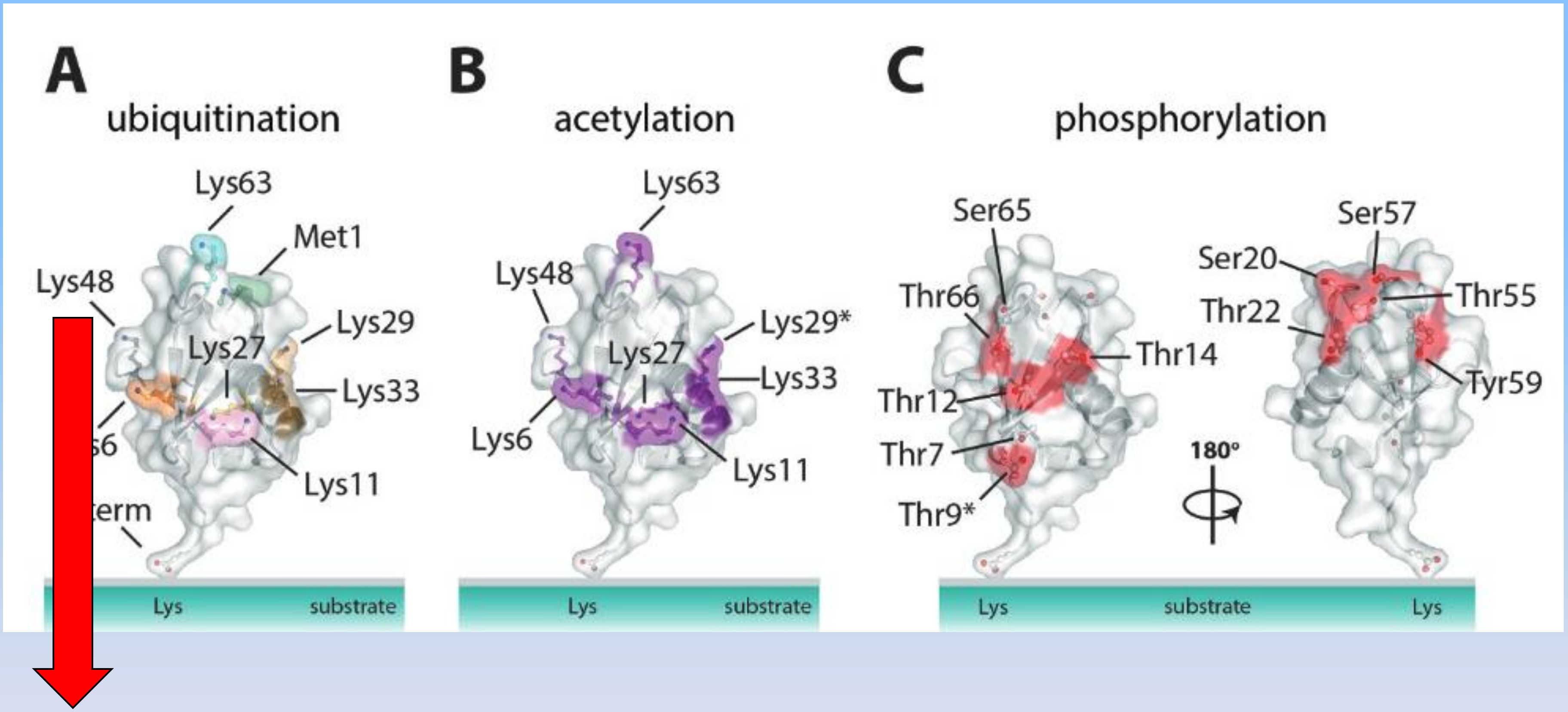
**Affiliation at the time of the award:** University of California, Irvine, CA, USA

**Prize motivation:** "for the discovery of ubiquitin-mediated protein degradation"

**Field:** Biochemistry

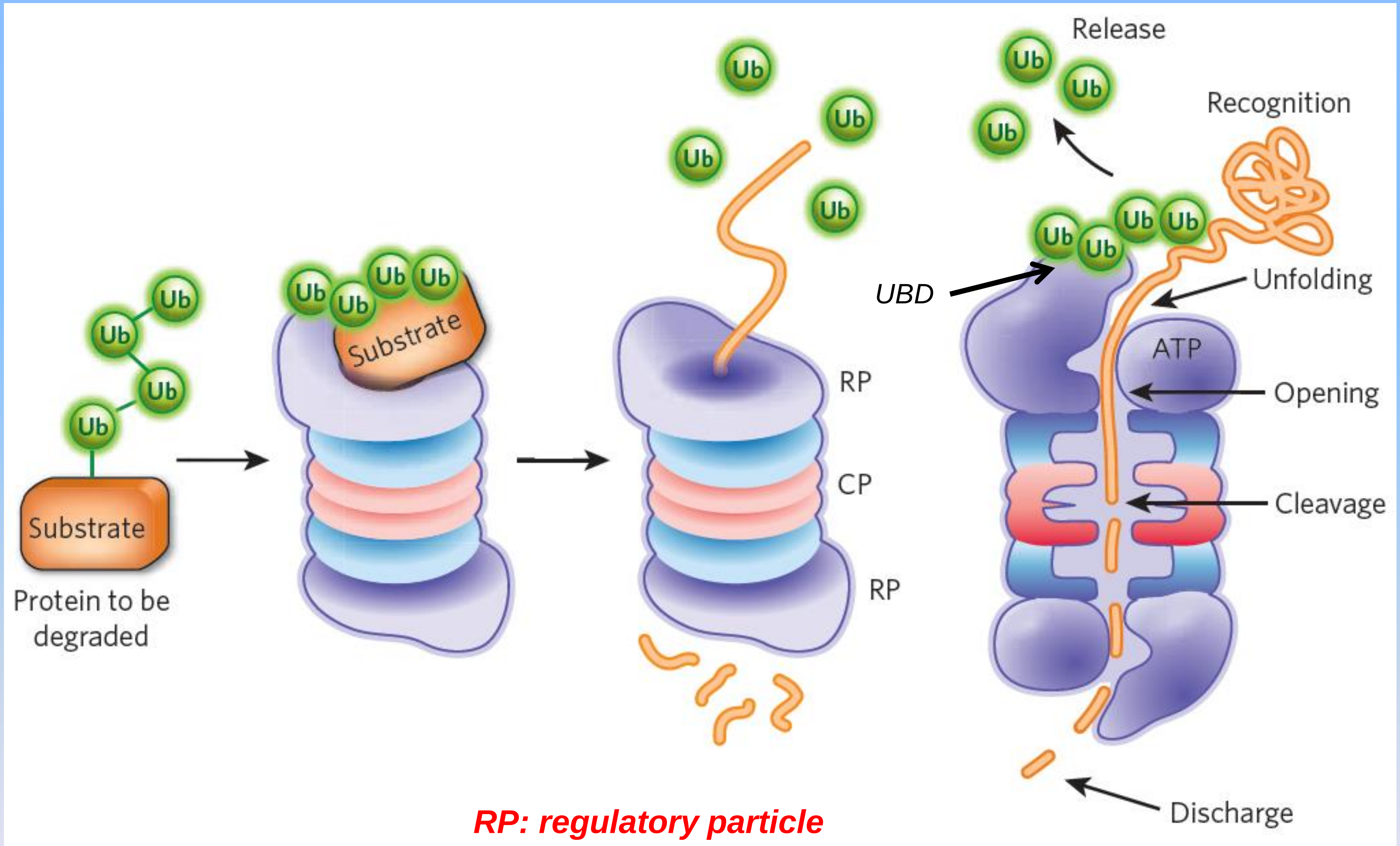
*http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2004/*

# *Ubiquitin Modifications*




***~50% of all modifications***
***Target proteins to the proteasome***

# Lys48 Poliubiquitination: Proteasome Degradation

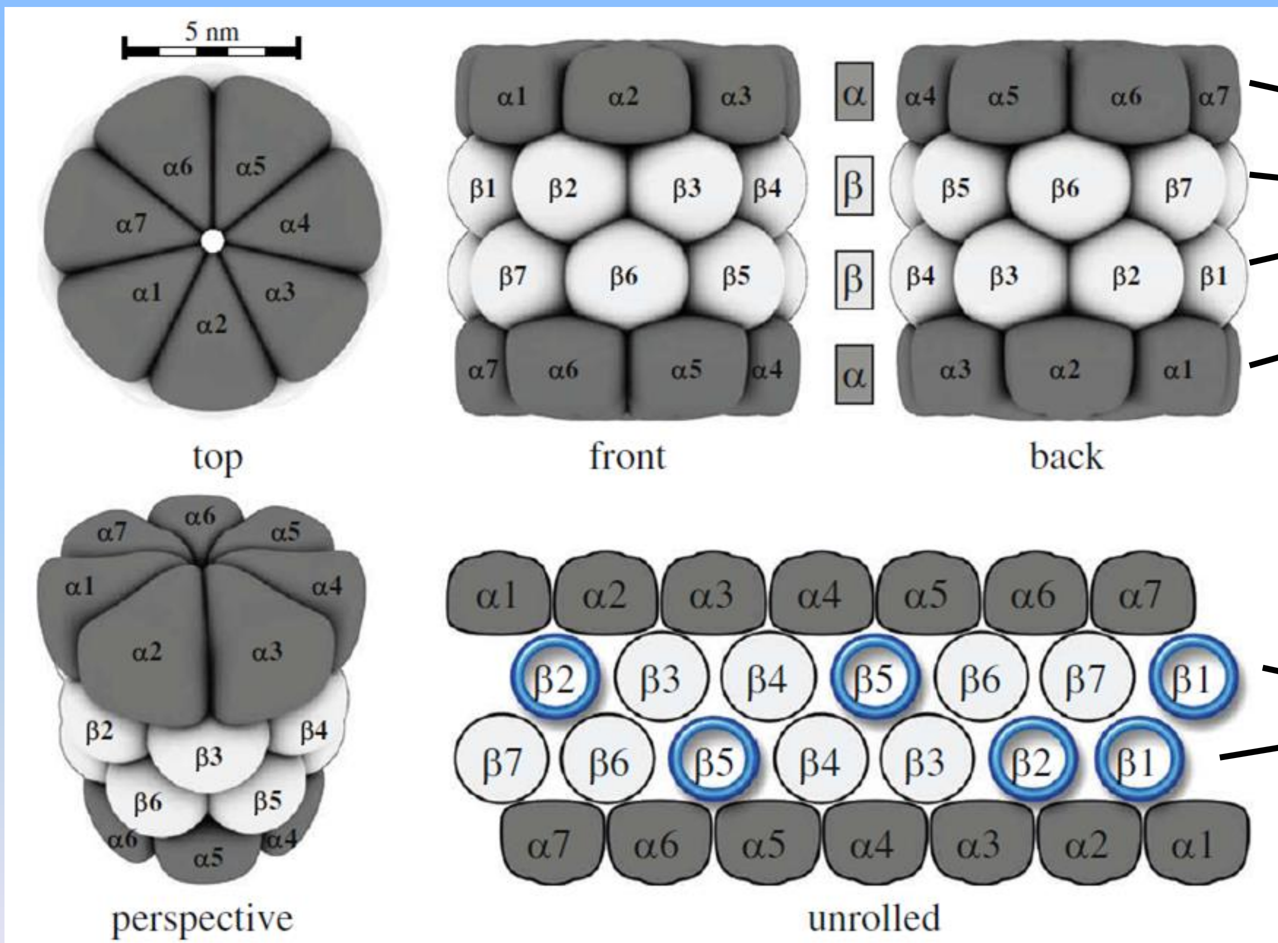



**RP: regulatory particle**
**CP: core particle (20S)**
**UBD: ubiquitin-binding domain**

*Adapted from: Hochstrasser, Nature 458(7237):422-9, 2009.*

# *Proteasome 20S (20S Core)*

*Present in all eukariotic and some prokariotic organisms*




***2 β rings***

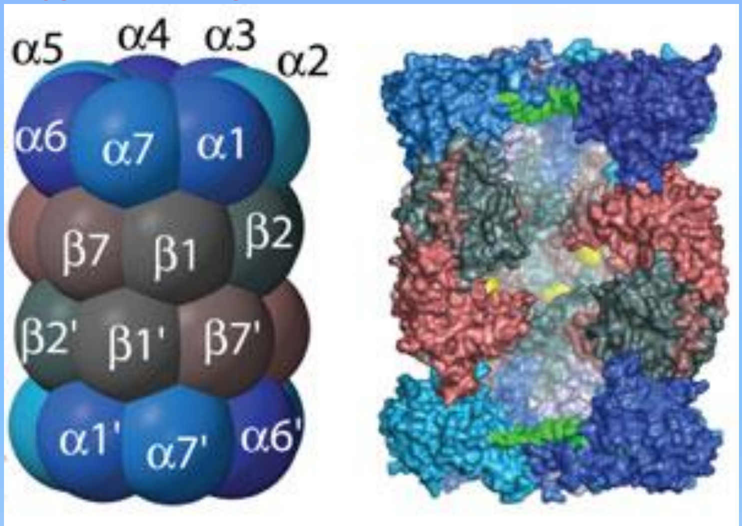
***2 α rings***

***β1, β2, β5 subunities: proteolitic activity***

*Jung et al., Molecular Aspects of Medicine, 30: 191-296, 2009.*

# Types of Proteasomes

http://picsdigger.com/image/6d0c4b8c/




***Cylindrin***

***Macroxyproteinase***

***Multicatalytic proteinase complex***

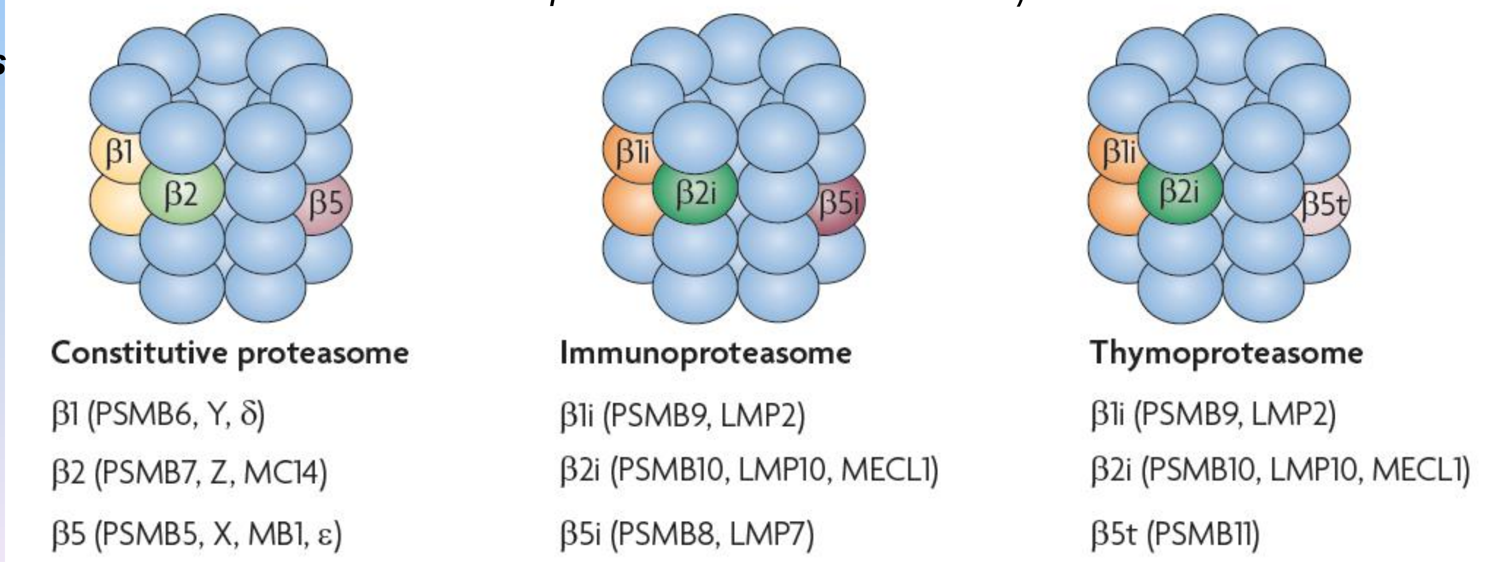
***Prosome***

***2-35 aa peptides***

***Peaks of:***
***2-3 aa***
***8-10 aa***
***20-30 aa***

*Optimized for 8-10 aa*

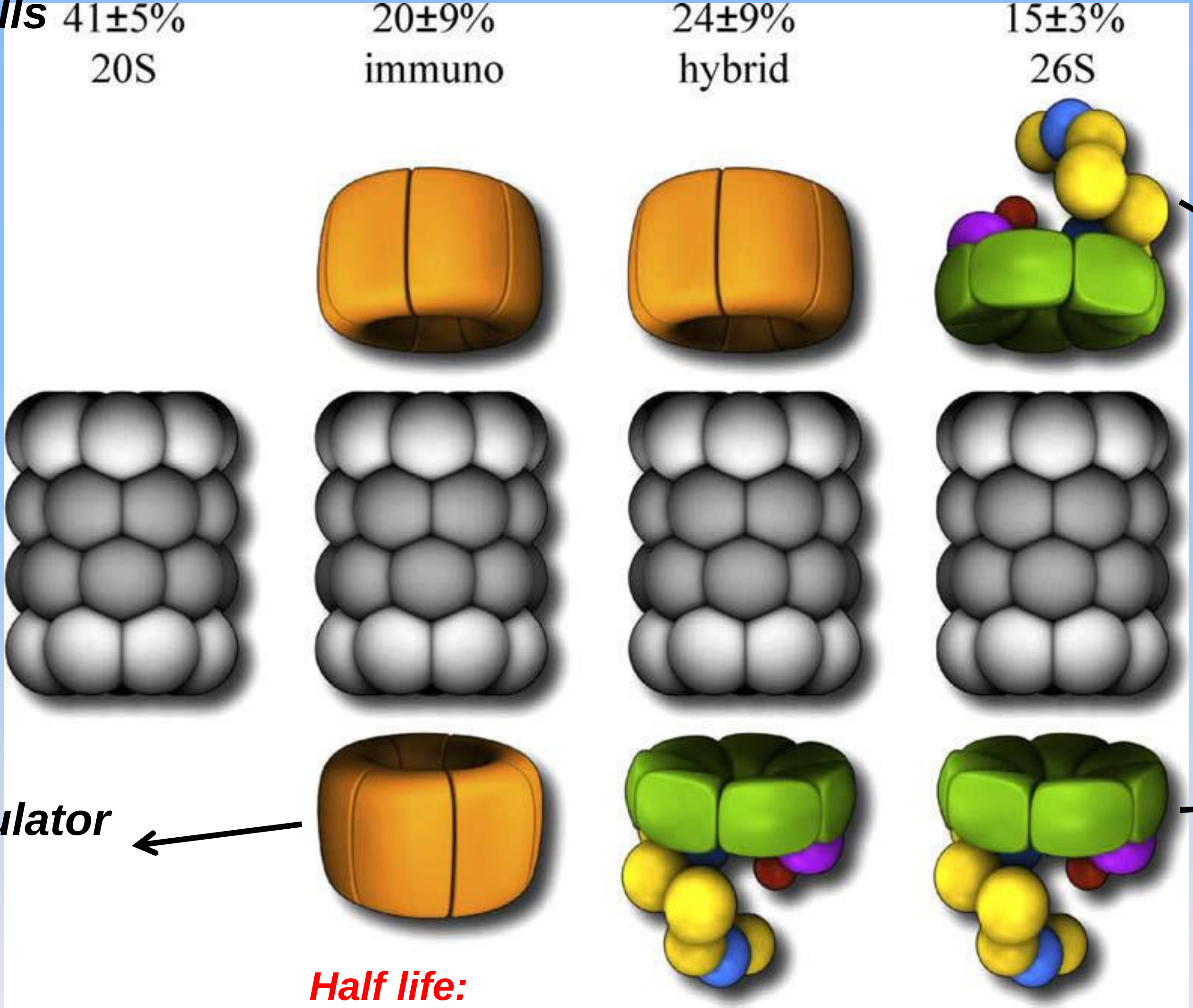
*β5t KO: less CD8+ T cells*




*Groettrup et al., Nat. Rev. Immunol., 10(1);73-8, 2010.*

# Proteasome Regulators

HeLa cells




MW: 2 MDa

Ubiquitin binding region

19S regulator

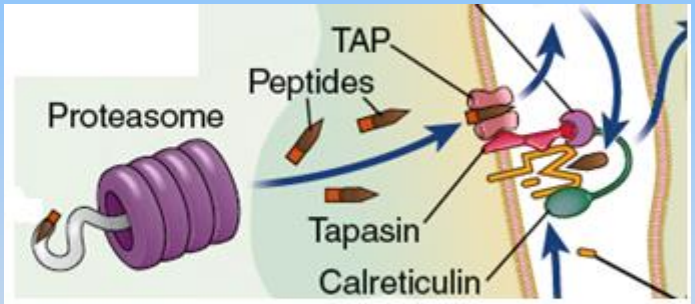
11S regulator (PA28)

Half life: 27 hours

Half life: 8-12 days

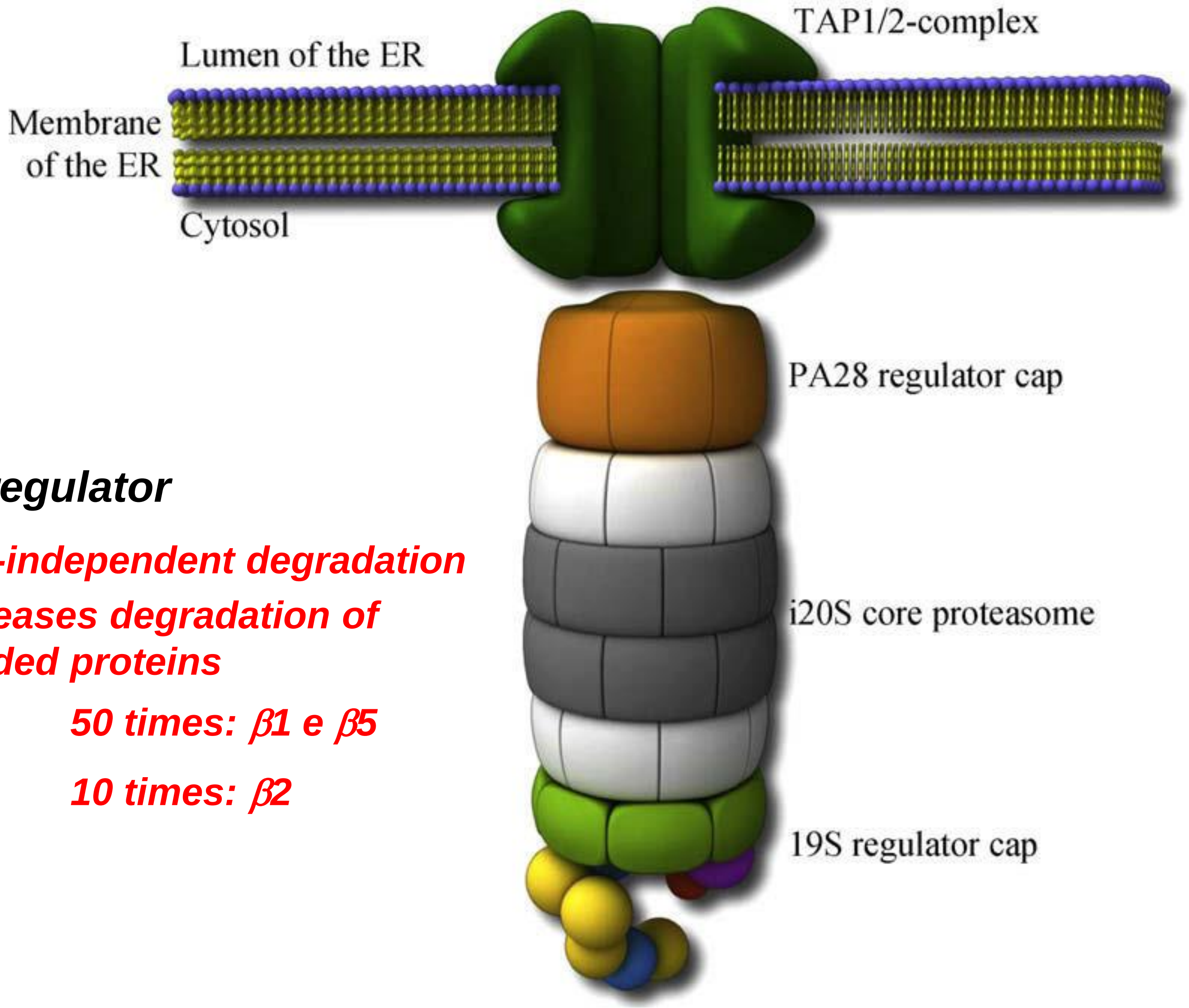
Jung et al., Molecular Aspects of Medicine, 30: 191-296, 2009.

# MHC Class I Pathway




Abbas, Lichtman, Pillai, 10th. Edition, 2021.

# Molecular Coupling Proteasome-TAP 1/2




***11S regulator***

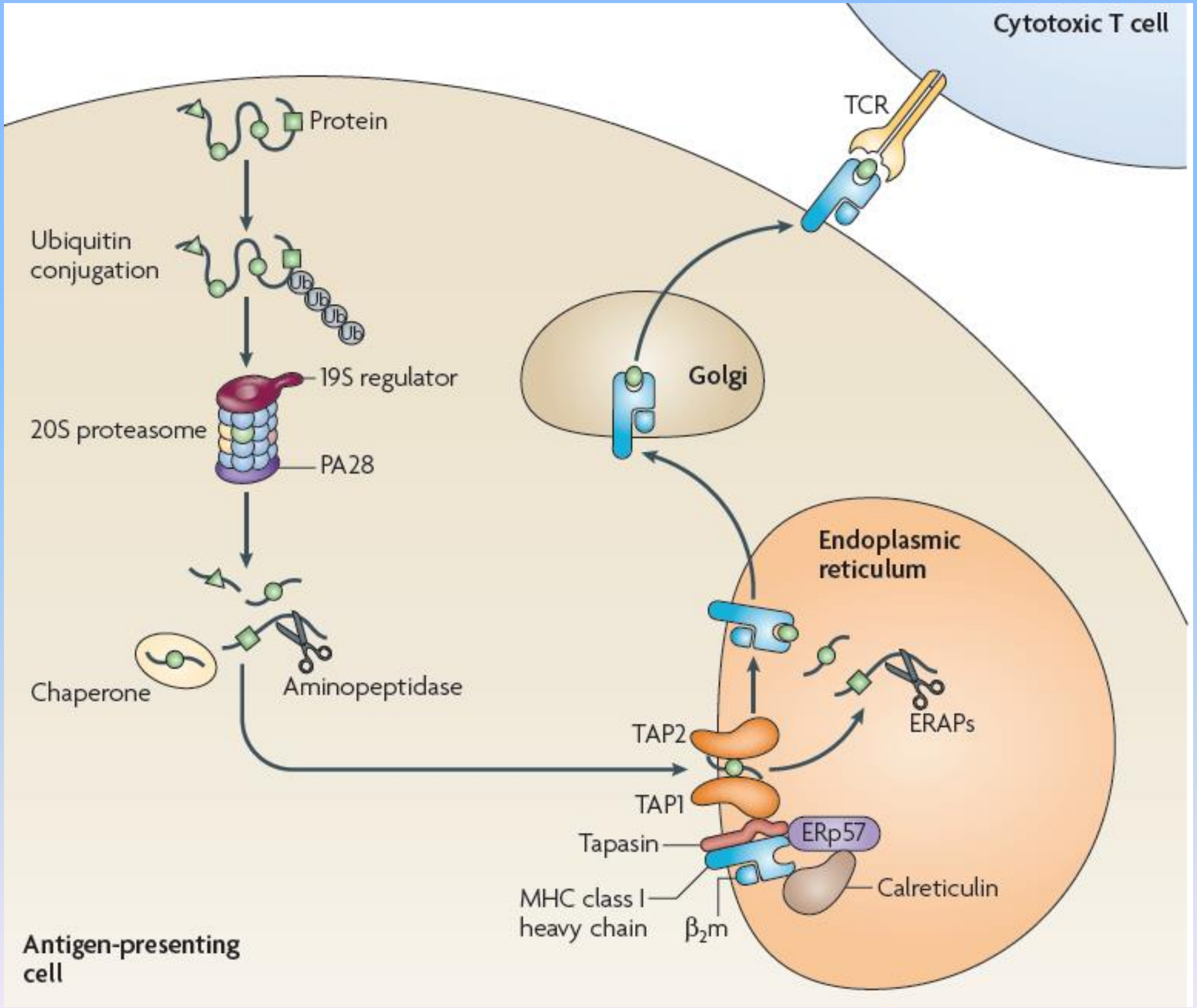
- ***ATP-independent degradation***
- ***Increases degradation of unfolded proteins***

***50 times: $\beta$1 e $\beta$5***

***10 times: $\beta$2***

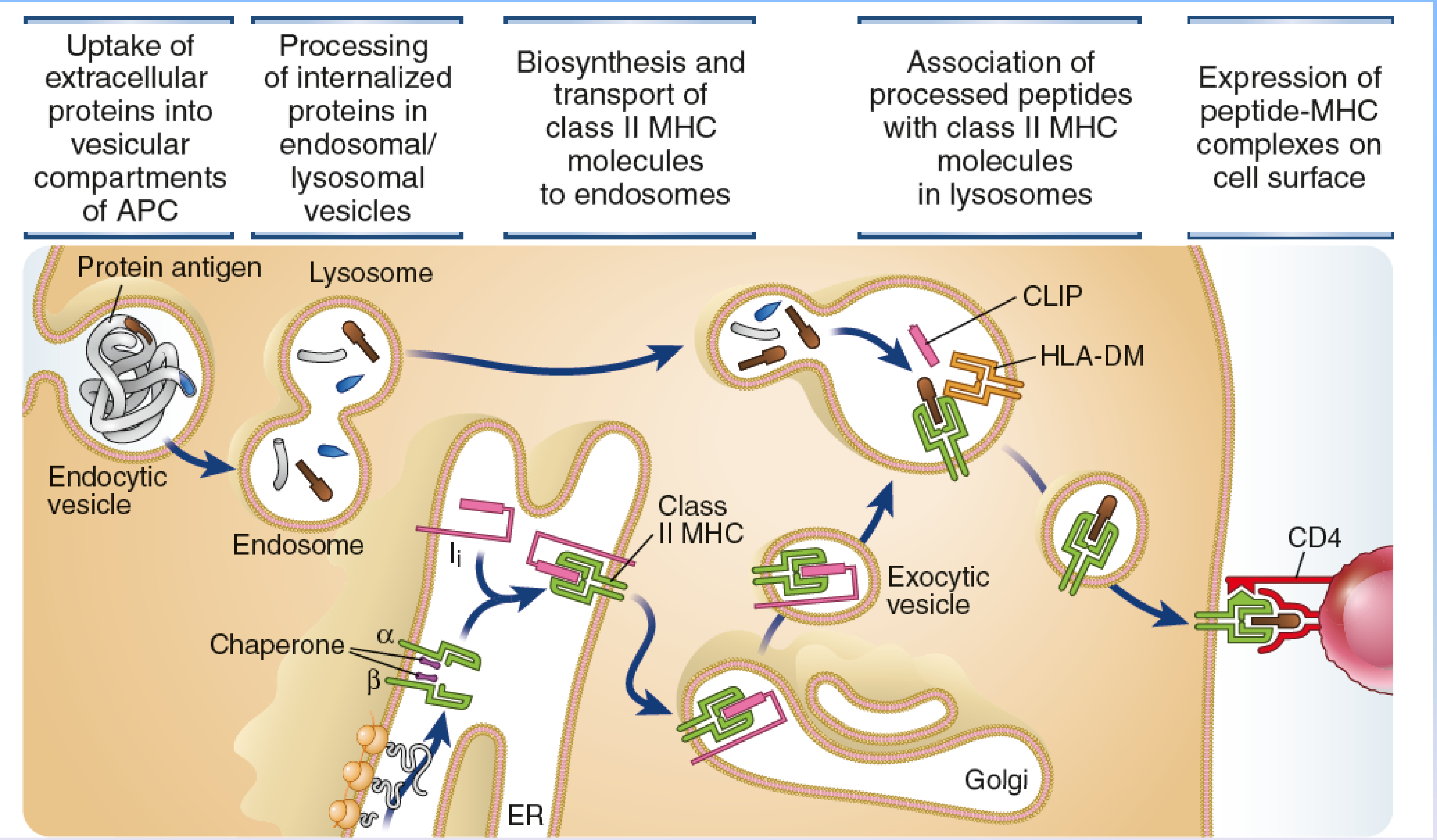
*Jung et al., Molecular Aspects of Medicine, 30: 191-296, 2009.*

# Processing of "Endogenous" Peptides



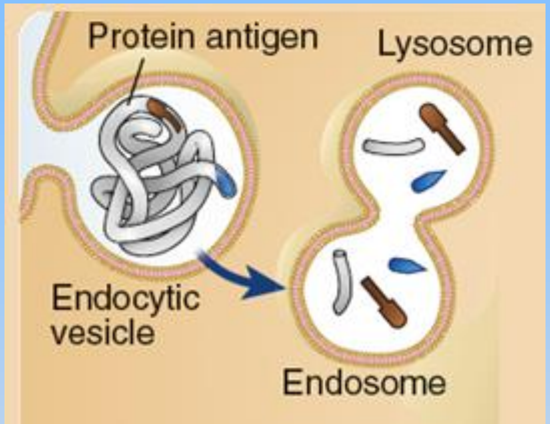

Groettrup et al., Nat. Rev. Immunol., 10(1);73-8, 2010.

# MHC Class II Pathway




*Abbas, Lichtman, Pillai, 10th. Edition, 2021.*

# MHC Class II Pathway




Abbas, Lichtman, Pillai, 10th. Edition, 2021.

# History of Lisosomal Proteases

**Lysosomotropic Agents** *(Biochem Pharmacol 23:2495-2531, 1974)*

*Proc. Natl. Acad. Sci. USA*
Vol. 79, pp. 175–178, January 1982
Immunology

**Decrease in macrophage antigen catabolism caused by ammonia and chloroquine is associated with inhibition of antigen presentation to T cells**

(macrophage–lymphocyte interactions/T-cell immunity/lysosomotropic agents/antibacterial immunity)

H. KIRK ZIEGLER* AND EMIL R. UNANUE†

Table 1. Inhibition of antigen presentation with $NH_4Cl$ and chloroquine

| Assay | Control, % | 10 mM $NH_4Cl$ Observed, % | 10 mM $NH_4Cl$ Δ,% | 0.1 mM chloroquine Observed, % | 0.1 mM chloroquine Δ,% |
|---|---|---|---|---|---|
| Antigen uptake* | 15 ± 1 | 13 ± 2 | 13 | 15 ± 2 | 0 |
| Antigen ingestion† | 66 ± 2 | 63 ± 2 | 5 | 67 ± 6 | −2 |
| Antigen catabolism‡ | 29 ± 4 | 13 ± 3 | 55 | 14 ± 6 | 52 |
| I-A expression§ | 54 ± 4 | 59 ± 2 | −9 | 56 ± 4 | −4 |
| T cell–macrophage binding¶ | | | | | |
| Before antigen handling | 70 ± 7 | 26 ± 8 | 63 | 30 ± 8 | 57 |
| After antigen handling | 84 ± 8 | 70 ± 11 | 17 | 64 ± 10 | 24 |

***Peritoneal macrophages***
***$^{125}$I-Listeria monocytogenes***

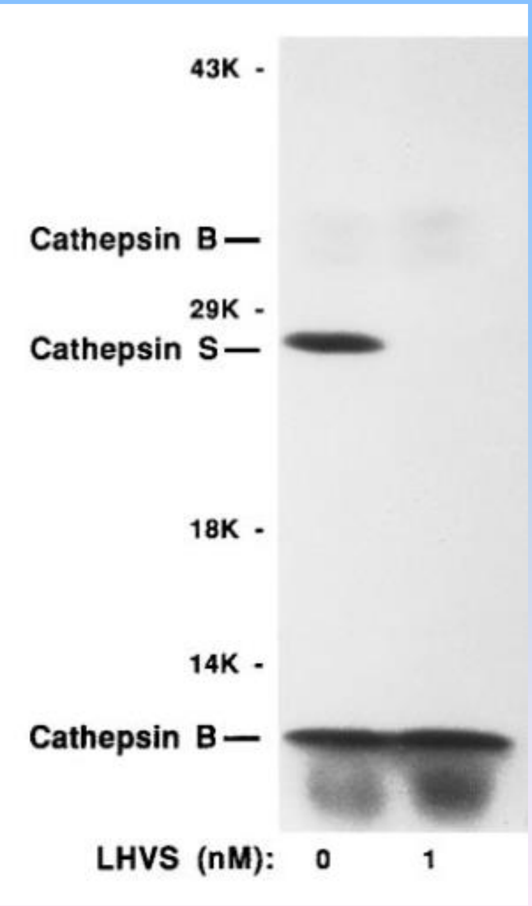
***Drug exposure (1 h):***
***- Before handling: starting 30 min before Listeria uptake***
***- After handling: 30 min after Listeria uptake***

# Cathepsin S: essential to antigen presentation

*Immunity, 1996, 4: 357-366.*

**Essential Role for Cathepsin S in MHC Class II–Associated Invariant Chain Processing and Peptide Loading**

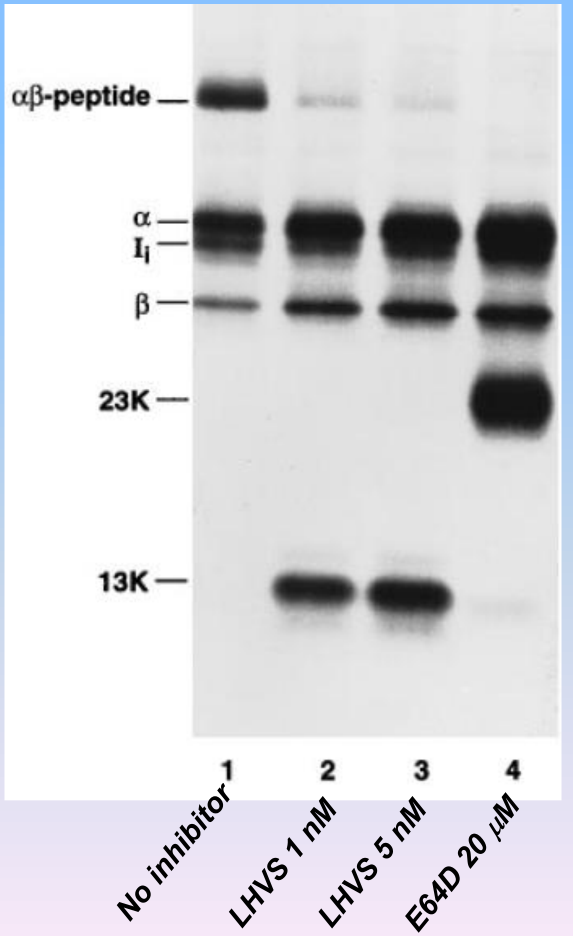
Richard J. Riese,* Paula R. Wolf,† Dieter Brömme,‡ Lisa R. Natkin,* José A. Villadangos,† Hidde L. Ploegh,† and Harold A. Chapman*




*[35][S]-methionine/cysteine*

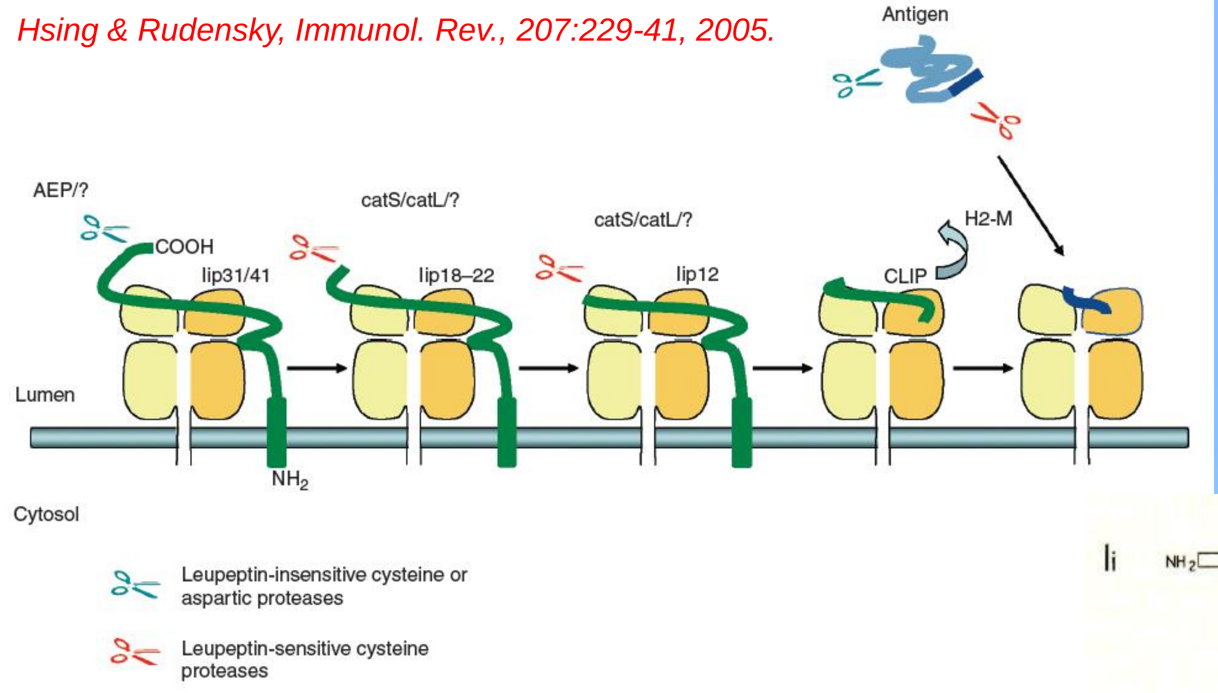
*Treatments:*
*- LHVS (Cathepsin S inhibitor)*
*- E64D (broad spectrum cystein protease inhibitor)*

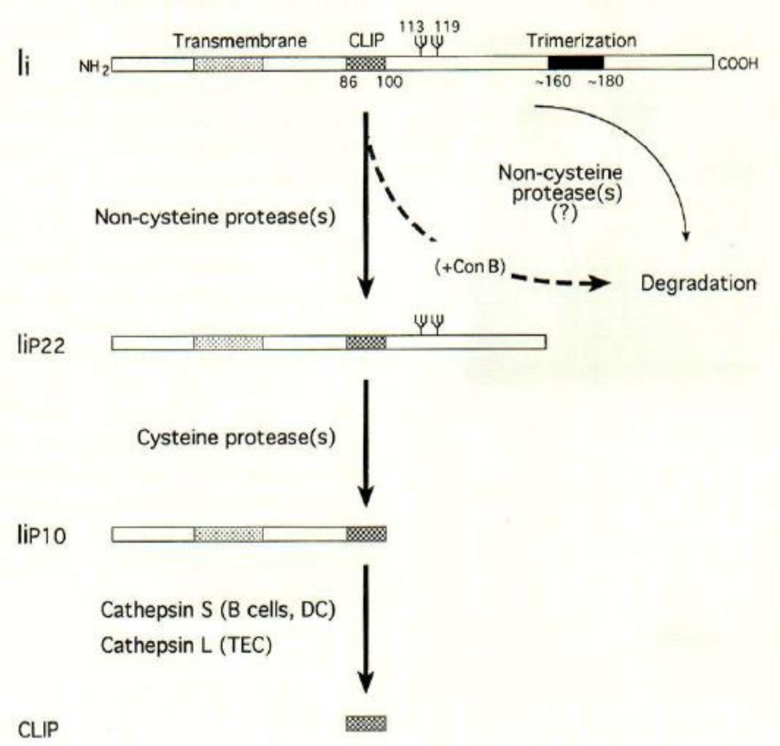
*Immunoprecipitation: anti-HLA-DR1*



# Invariant Chain Degradation

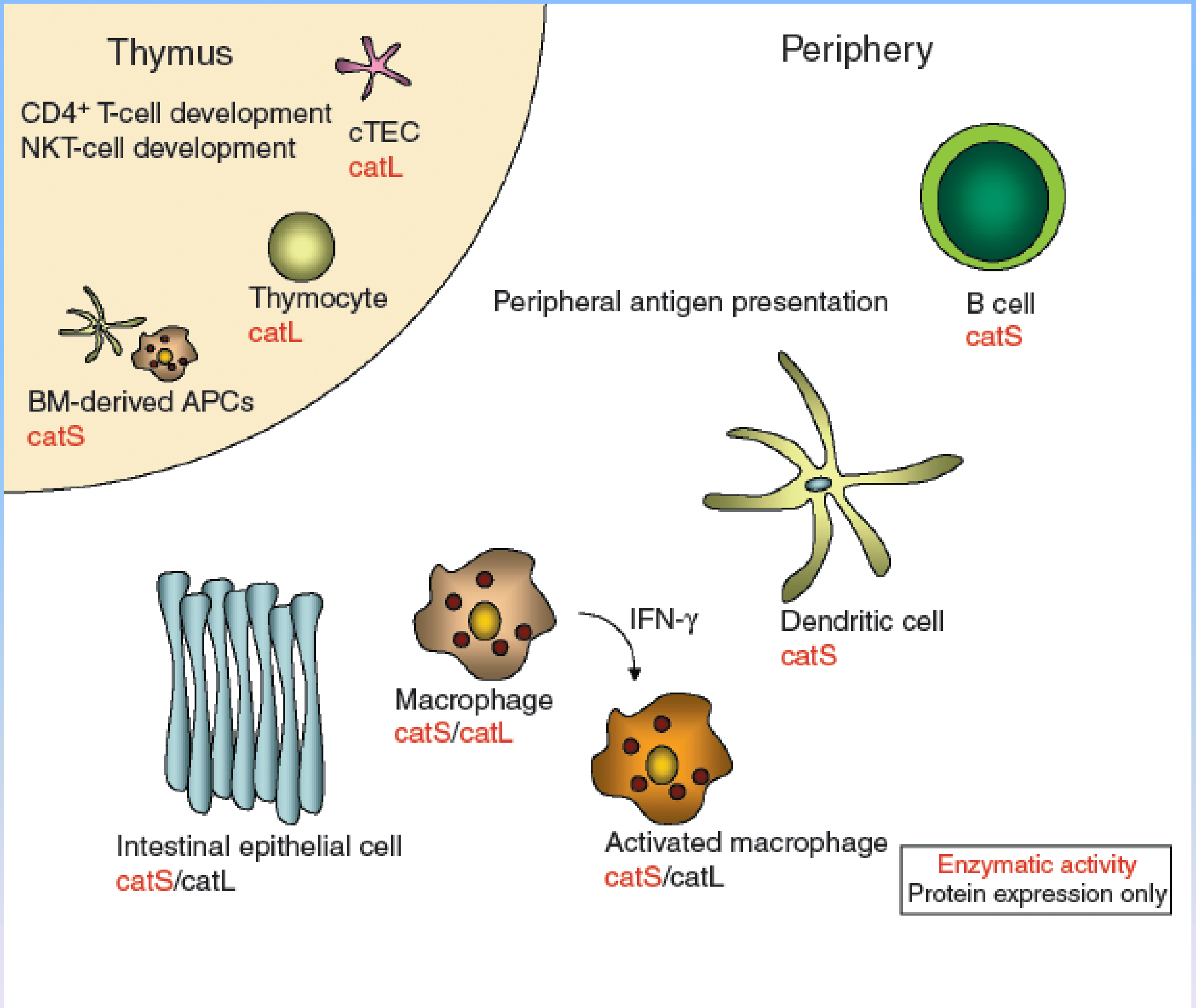
Hsing & Rudensky, Immunol. Rev., 207:229-41, 2005.



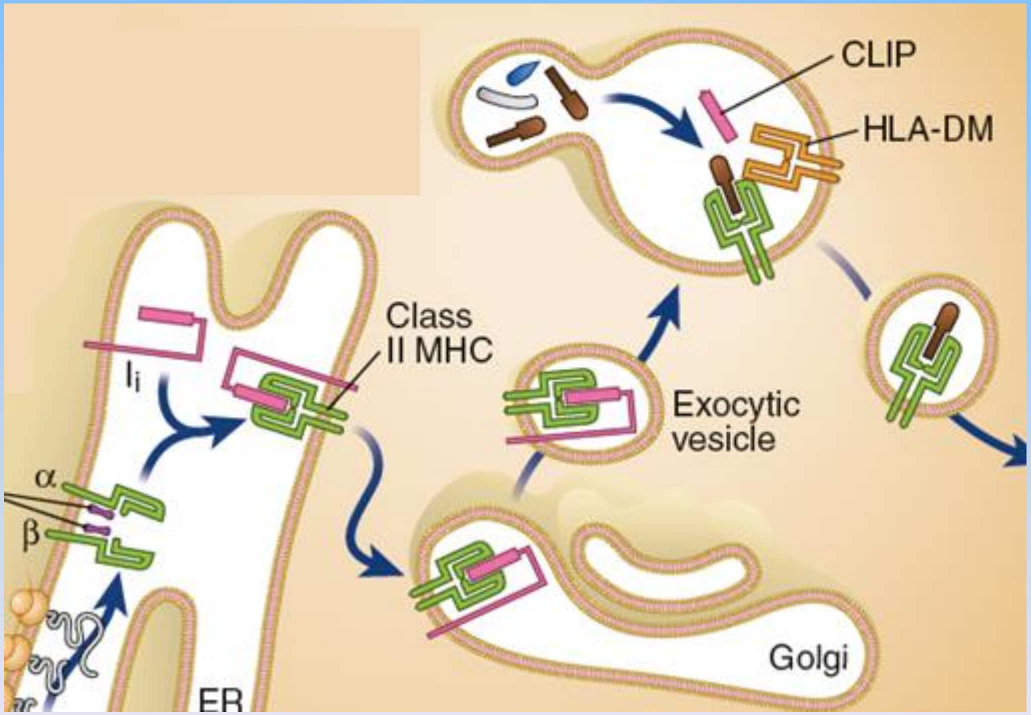

Villadangos et al., Immunological Reviews 172:109-20, 1999.

# Cathepsin S and L Expression by APCs



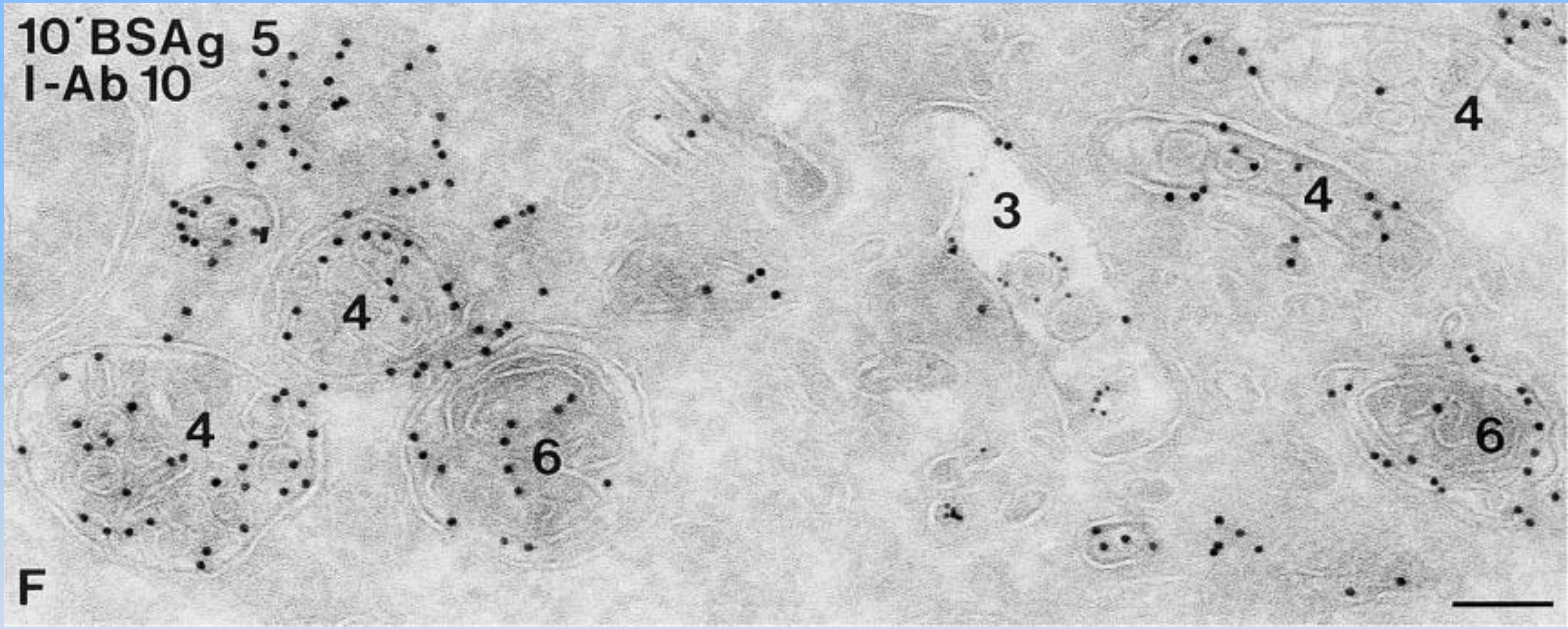

*Hsing & Rudensky, Immunol. Rev., 207:229-41, 2005*

# MHC Class II Pathway



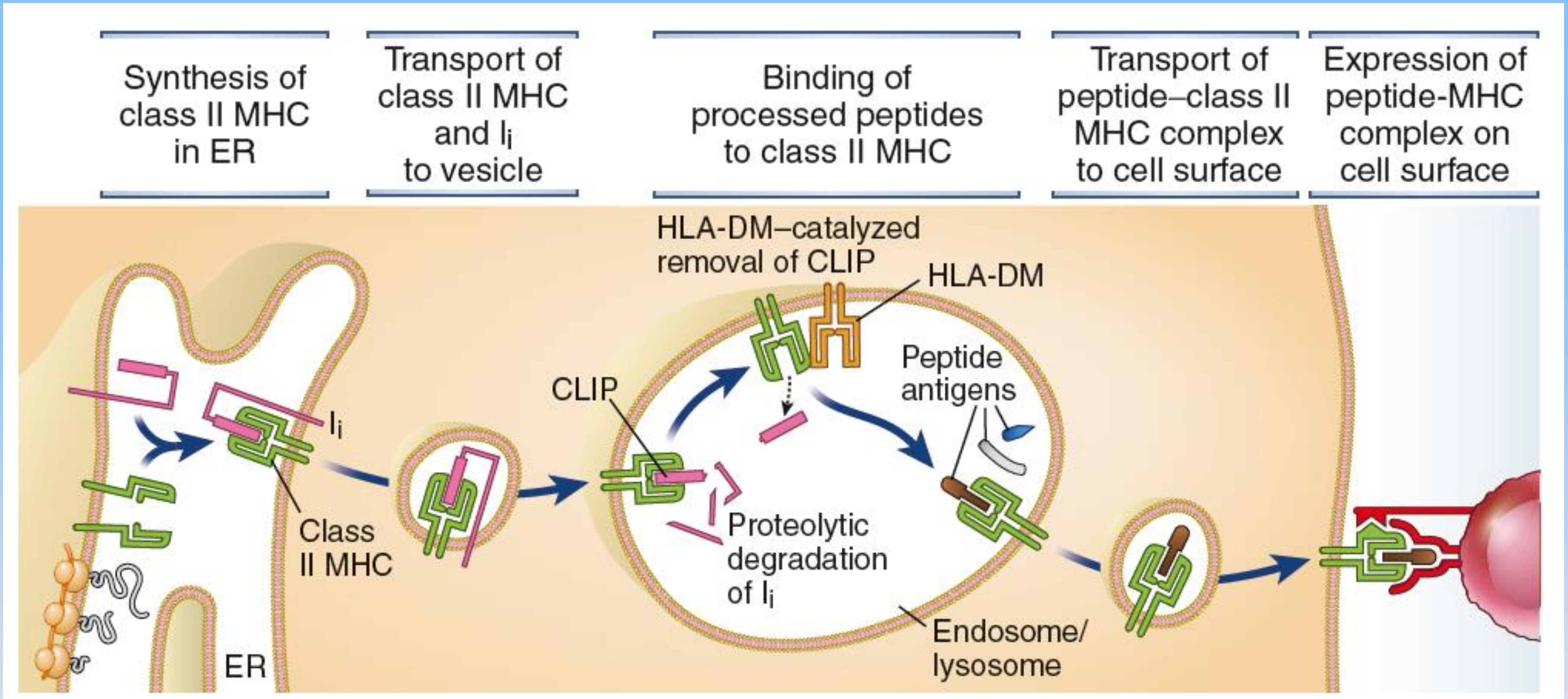

Abbas, Lichtman, Pillai, 10th. Edition, 2021.

# Class II Compartment (MIIC)




Kleijmeer et al., J. Cell Biol., 139:639-49, 1997

# Final Steps of MHC Class II Pathway



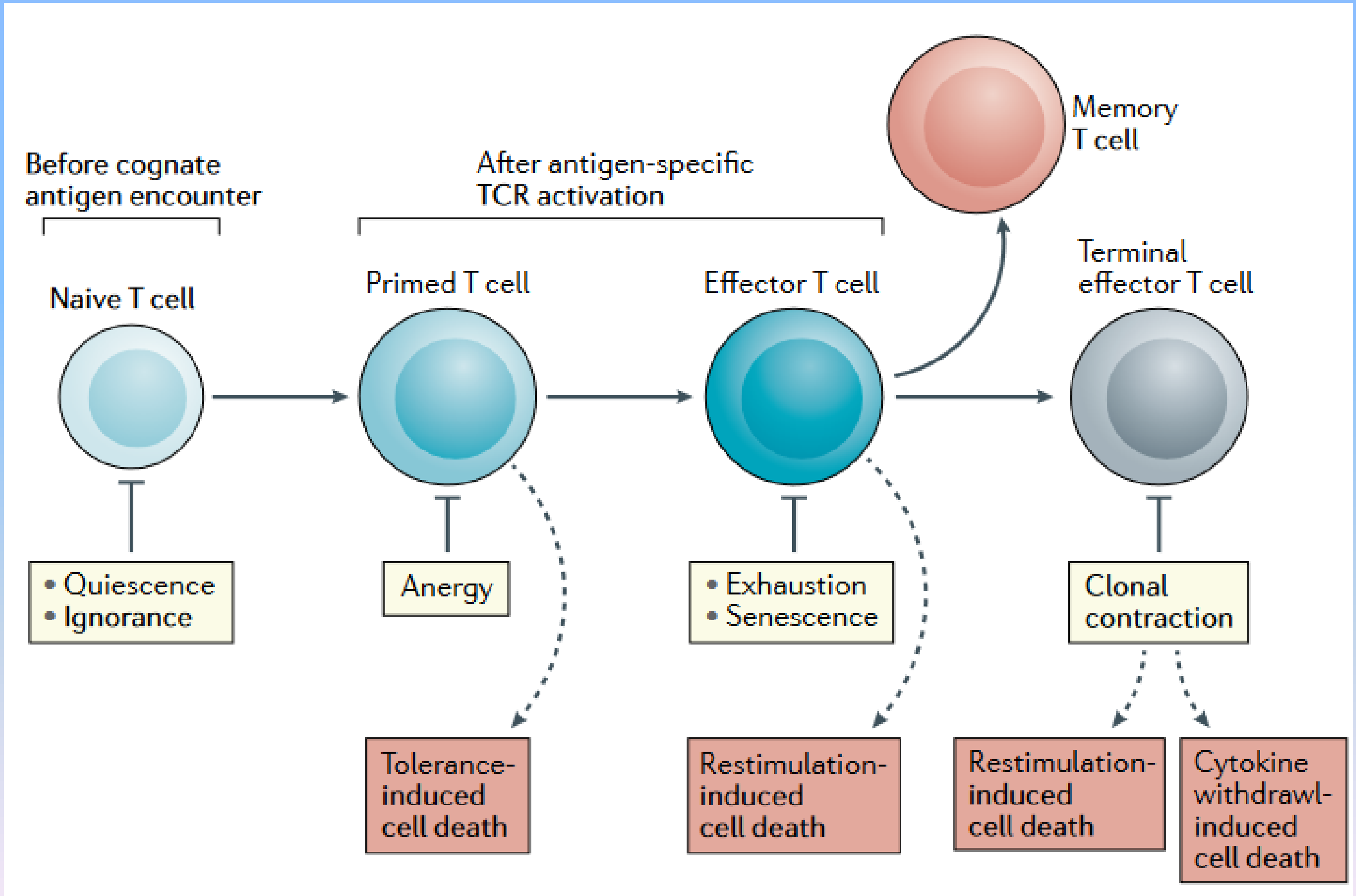

*Abbas, Lichtman, Pillai, 10th. Edition, 2021.*

# *Peripheral T cell Tolerance: Facts*

- *Thymic deletion of self-reactive T cells is only ~60–70% efficient*
- *Peripheral naive T cell repertoire contains a significant portion of low-avidity, self-reactive T cells*
- *Peripheral tolerance checkpoints include:*

1) *Mechanisms that act directly on the responding T lymphocyte (T cell-intrinsic mechanisms)*
2) *Mechanisms that depend on other cell subsets, such as regulatory T cells (Treg cells) and dendritic cells (T cell-extrinsic mechanisms).*

# Checkpoints for Peripheral T cell Tolerance




ElTanbouly & Noelle, Nat. Rev. Immunol., 21(4):257-267, 2021.

# *Checkpoints for Peripheral T cell Tolerance*

- ***Quiescence: sleeping?***
- ***Ignorance: it is there, but they just do not know.***
- ***Anergy: insufficient signals? The starting motor does not work…***
- ***Exhaustion: tired, they are done…***
- ***Senescence: just getting old!***
- ***Deletion: shot and killed!***

# Checkpoints for Peripheral T cell Tolerance

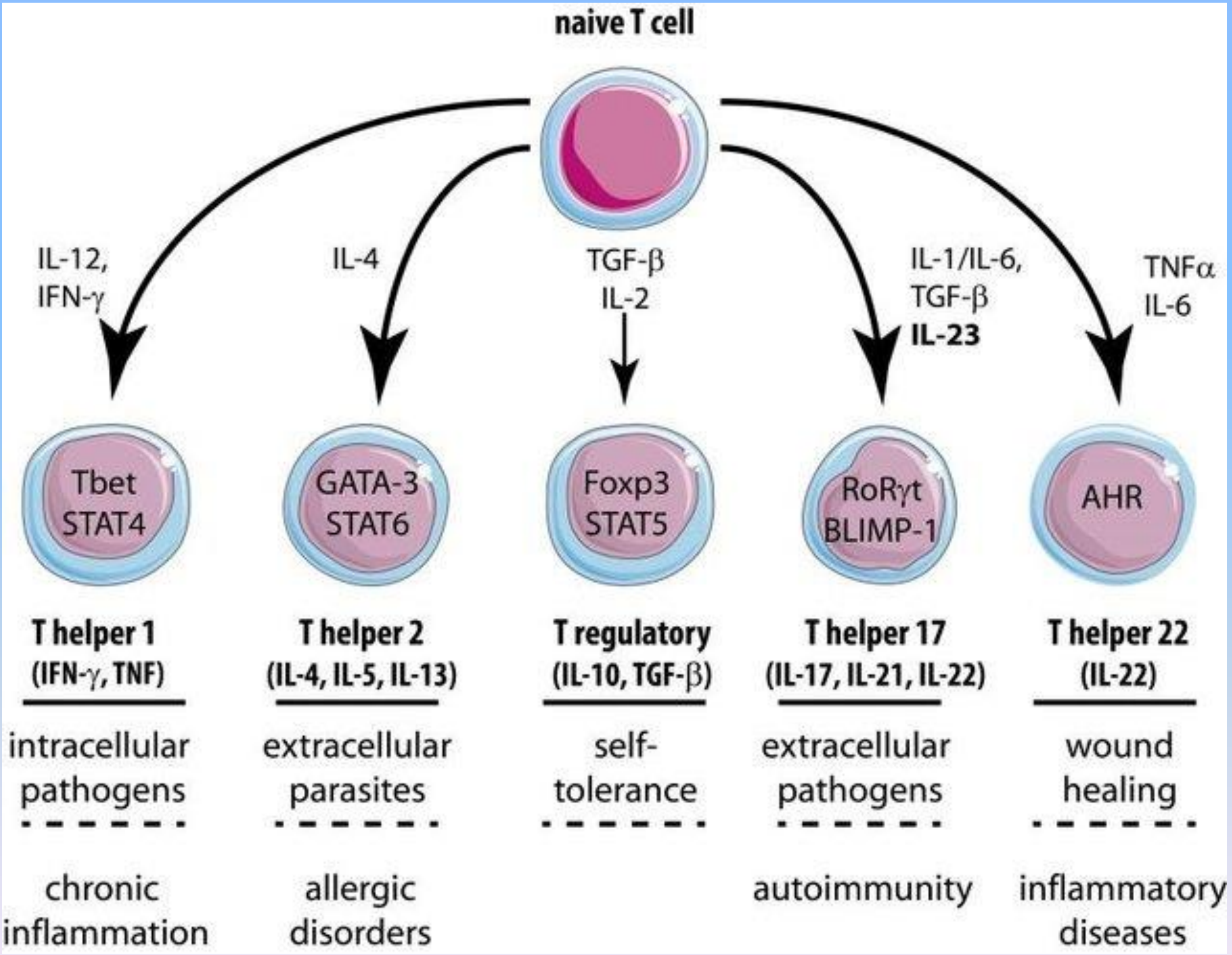
Table 1 | **Summary of the main regulators and markers of each tolerance checkpoint in T cells**

| Factor | Quiescence | Ignorance | Anergy | Exhaustion | Senescence | Deletional tolerance |
|---|---|---|---|---|---|---|
| Surface receptors | TGFβR1 (REF.[13])<br>VISTA[17] | Unknown | CD73 (REFS[78,79])<br>FR4 (REFS[78,79])<br>LAG3 (REF.[85])<br>NRP1 (REF.[79]) | PD1 (REF.[177])<br>TIGIT[178]<br>LAG3 (REF.[179])<br>TIM3 (REF.[180]) | NKG2D[133]<br>IFNα/IFNAR[119] | FAS (also known as CD95)[160,161]<br>TNFR1(REFS[164,181])<br>TRAILR1 and TRAILR2 (REFS[182,183]) |
| Signalling molecules | BTG1/BTG2 (REF.[18])<br>TSC1/TSC2 (REFS[31,32]) | Unknown | DGKα[62]<br>CBLB[67]<br>GRAIL[184]<br>ITCH[185,186] | SHP1 (REF.[187])<br>SHP2 (REFS[187,188])<br>PTPN2 (REF.[189]) | TAB1 (REF.[129])<br>Sestrin 2 (REF.[133]) | CASP8 (REF.[161])<br>BID[190]<br>BIM (also known as BCL-2L11)[145,146] |
| Transcription factors | KLF2 (REFS[20,22])<br>FOXO1 (REFS[26–28])<br>RUNX1 (REFS[29,30])<br>TOB1 (REFS[14,15])<br>FOXP1 (REFS[191,192]) | Unknown | NFAT1 (REF.[64])<br>EGR2 (REFS[66–68])<br>EGR3 (REF.[67])<br>NR4A1 (REF.[72])<br>TOB1 (REF.[15]) | IRF4 (REF.[193])<br>NR4A1 (REF.[103])<br>GATA3 (REF.[194])<br>TOX[103–106]<br>BATF[102]<br>BLIMP1 (REF.[195])<br>EOMES[196] | – | – |

DGKα, diacylglycerol kinase-α; IFNα, interferon-α.

ElTanbouly & Noelle, Nat. Rev. Immunol., 21(4):257-267, 2021.

# *Development of T cell subsets*




*Adapted from: Golubovskaya & Wu, Cancers, 8(3):36, 2016.*

# Regulatory T cell subsets

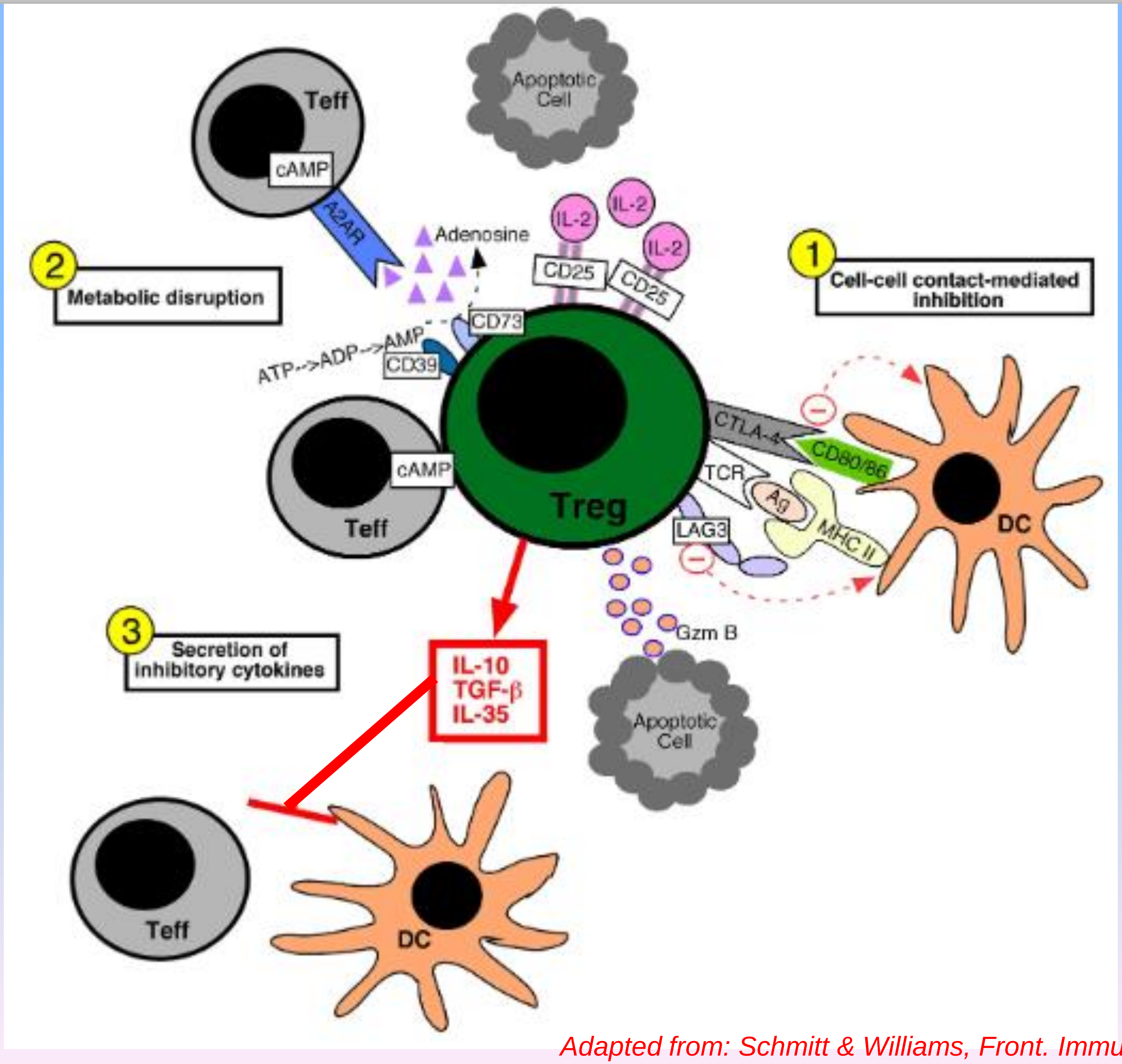
**TABLE 1** | Different Treg subsets identified in human and/or mouse.

| Cell population | Human | Mouse | Reference |
|---|---|---|---|
| $CD4^+$ $CD25^+$ $Foxp3^+$ | ✓ | ✓ | (5, 6) |
| $CD4^+$ $Foxp3^{low}$ $CD45RA^+$ | ✓ | ✗ | (12) |
| $CD4^+$ $Foxp3^{high}$ $CD45RA^-$ | ✓ | ✗ | (12) |
| $CD4^+$ $Foxp3^-$ $IL10^+$ (Tr1) | ✓ | ✓ | (37) |
| $CD4^+$ $Foxp3^-$ $TGF\beta^+$ (Th3) | ✓ | ✓ | (38) |
| $CD4^+$ $Foxp3^-$ $IL35^+$ (iTr35) | ✓ | ✓ | (39) |
| $CD4^+$ $Foxp3^-$ $IL10^+$ $TGF\beta^+$ (Treg of B cells) | ✗ | ✓ | (40) |
| $CD8^+$ $Foxp3^+$ and/or $CD28^-$ and/or $CD25^+$ | ✓ | ✗ | (41–43) |
| $CD8^+$ $CD45RA^+$ $CCR7^+$ $Foxp3^+$ | ✓ | ✗ | (44) |
| $CD8^+$ $CD45RC^{low/-}$ | ✓ | ✓ | (45) |
| $CD8^+$ $CD122^+$ | ✗ | ✓ | (46) |
| $CD8^+$ HLA-$DR^+$ | ✓ | ✗ | (47) |
| $CD8^+$ HLA-$E^+$ | ✓ | ✗ | (48) |
| $CD8^+$ Qa-$1^+$ | ✗ | ✓ | (48) |
| γδ T cells | ✓ | ✓ | (49) |
| NKT | ✓ | ✓ | (50) |

*Treg cells are categorized based on CD4 or CD8 surface markers expression.*

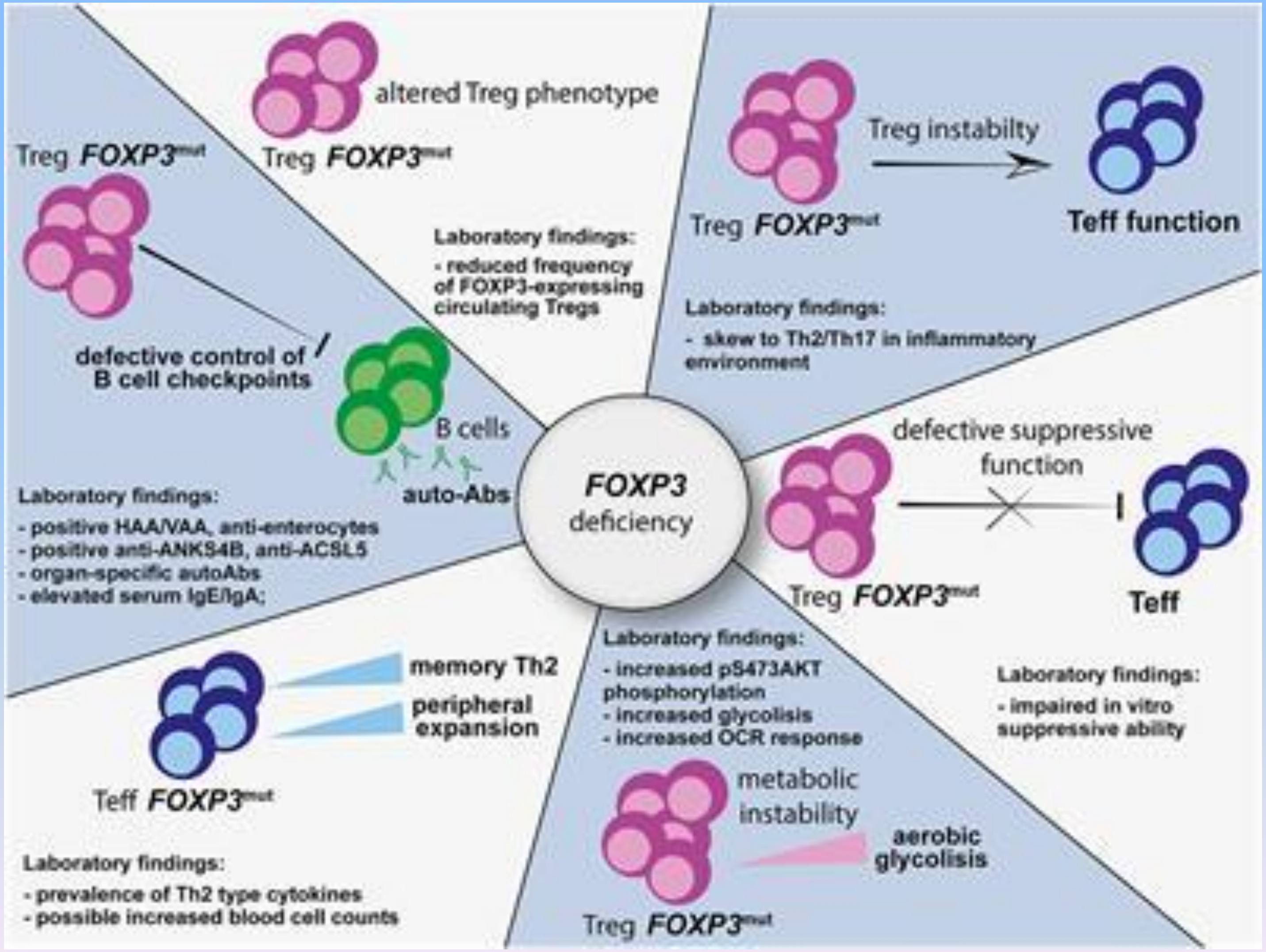
Rocamora-Reverte et al., Front. Immunol., 11:616949, 2021.

# Mechanisms of Suppression by Regulatory T cells




*Adapted from: Schmitt & Williams, Front. Immunol., 4:152., 2013.*

# *Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome*




*Barzaghi & Passerini, Front. Pediatr., 9:612760, 2021.*

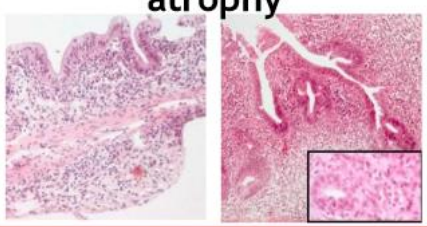
# *Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome*

**Classical triad of symptoms**

**Dermatitis**

**Type 1 diabetes**

**Enteropathy with villous atrophy**

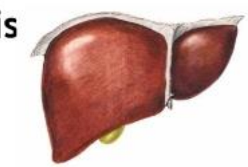


**Alopecia**

**Autoimmune thyroiditis**

**Recurrent infections**

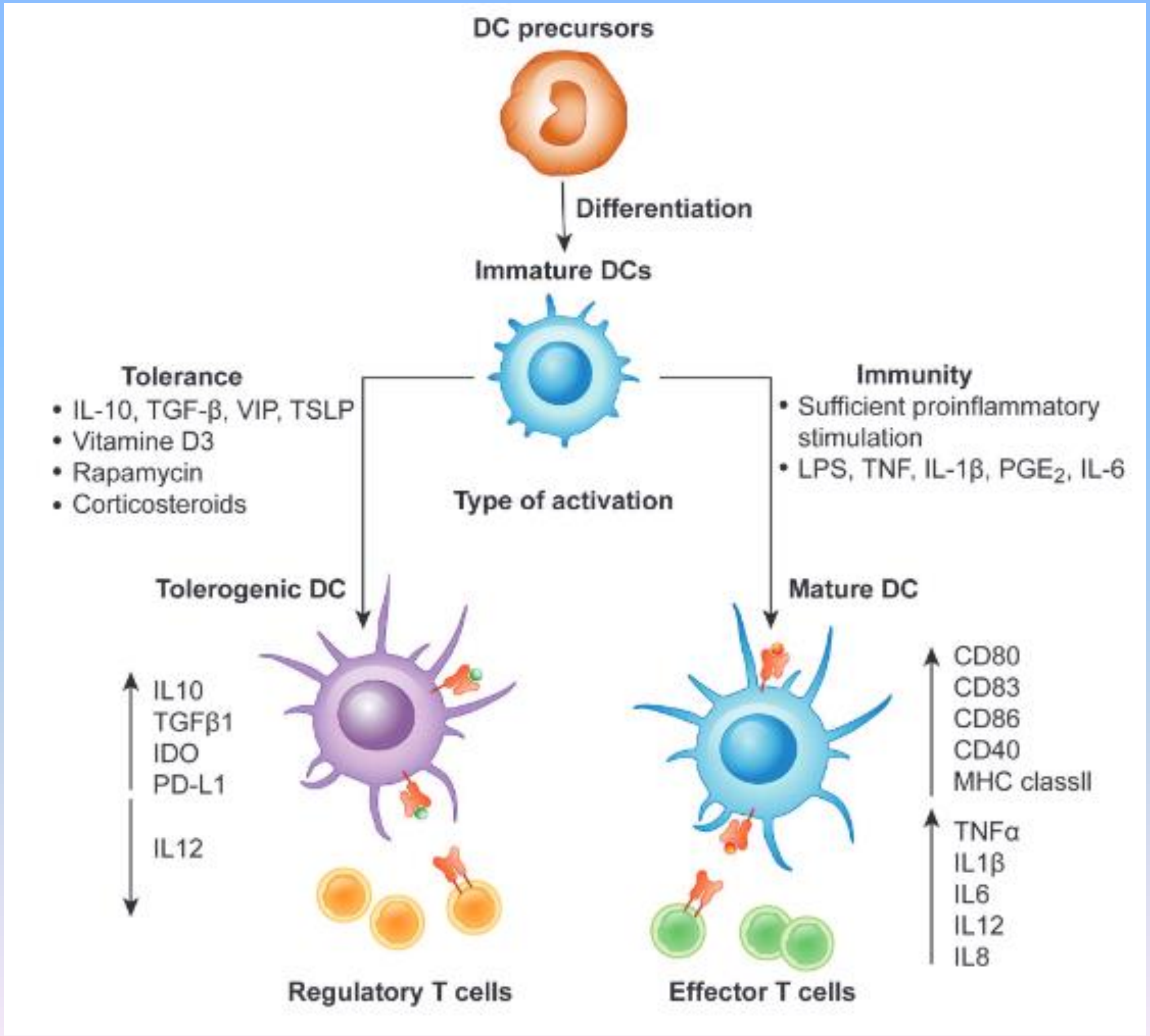
**Autoimmune hepatitis**



**Additional symptoms**

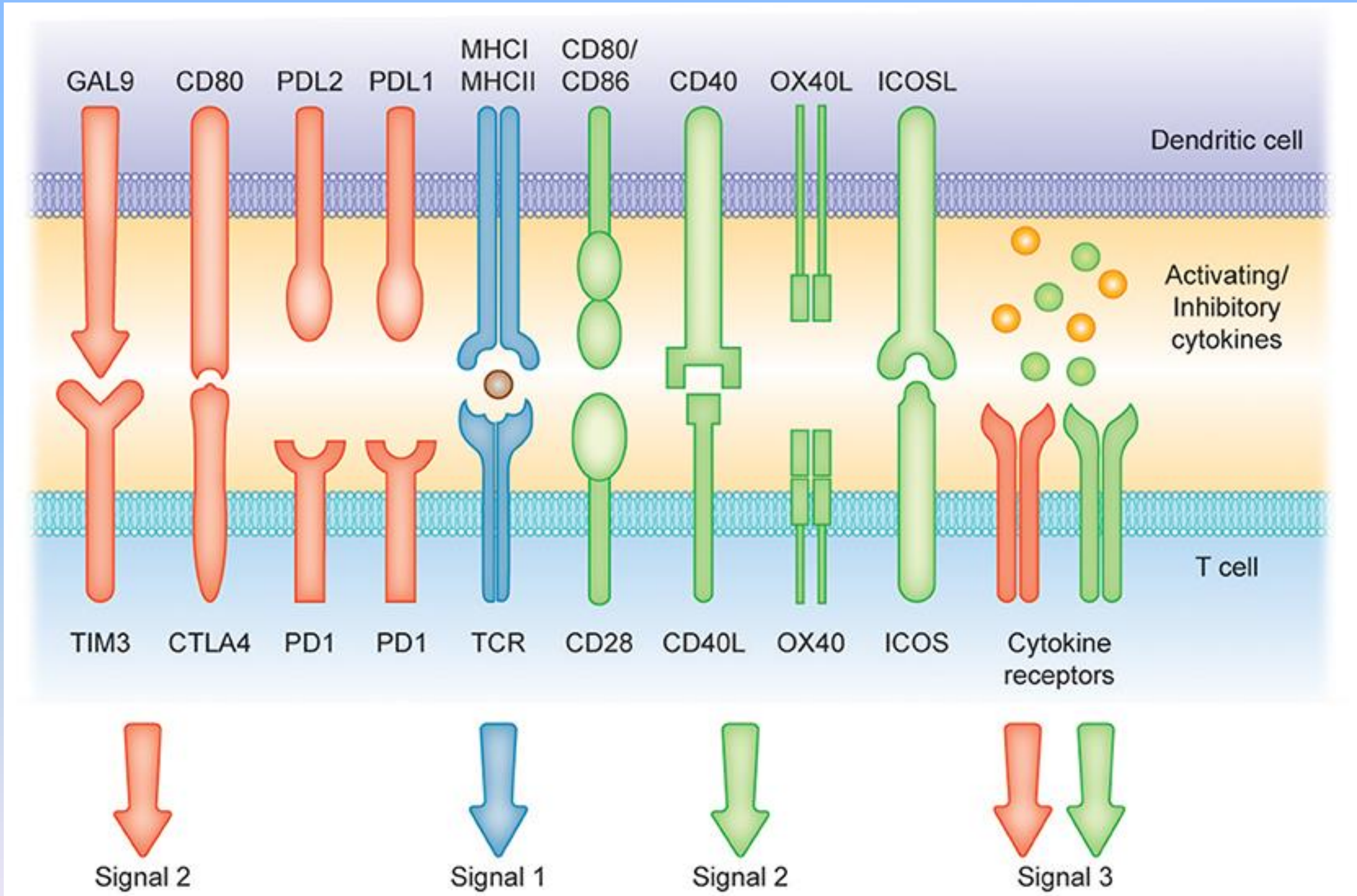
*http://docplayer.net/1255997-Foxp3-il-peacekeeper-del-sistema-immunitario.html*

# *Dendritic cells: tolerance versus immunity*




*Fucikova et al., Front. Immunol., 10:2393, 2019.*

# Tolerance or immunity depend on the molecular Interaction with T cells

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# Extrathymic Role for AIRE in peripheral DCs

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## Extrathymic *Aire*-Expressing Cells Are a Distinct Bone Marrow-Derived Population that Induce Functional Inactivation of $CD4^+$ T Cells

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