MHC molecules & Ag presentation to TLs

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Principal function of MHC molecules

- Bind, display, & present Ag peptides by other cells (*by APCs to CD4+ HTLs, & *by all nucleated cells to CD8+ CTLs) for Ag-specific recognition & binding with TCR → **Activation of (naïve) TLs** = initial, essential, crucial, & critical step in initiation, induction, & stimulation of (primary) TCM-IR → clonal expansion (proliferation), & differentiation of naive TLs into functioning effector, & memory TLs.

Challenges for TLs to function, & Features of Ags Recognized by TLs	
1- **Naive TLs** *__Generated__ in thymus acc/to Clonal Selection Hypothesis before Ag exposure. *with predetermined multiple Ag specificities (receptors specific for any Ag). *__Small pool__; very small # (1 in 10^5 or 10^6 Ls) vs TLs have to combat MOs at any site in body → Normal continuous __Recirculation__ in TL-zones of SPLOs (LNs).	__DCs__ (widely anatomically located) capture & display Ags & migrate to same TLs zones of SPLOs (__Colocalization__) → **Maximum Chance** of TLs with particular specificity to find relevant Ag peptide to recognize.
2- TLs recognize __ONLY peptides__ & NO other molecules (→ ONLY foreign protein Ags induce TCM-IRs). *vs BLs recognize proteins, peptides, NAs, CHOs, lipids, & small chemicals (→ protein & non-protein Ags induce humoral IRs).	__Processing__ (proteolysis, proteolytic digestion, degradation, peptide bond cleavage) of foreign protein Ags → loss of protein conformation (unfolding), & generation of short linear Ag peptides.
3- TCRs for Ags recognize specific AA sequences of __short linear peptides.__ *vs NOT conformational epitopes of large foreign protein Ags.	
4- __TCRs for Ags__ recognize ONLY __cell-associated__ Ags, & interact with other host cells (either APCs, or any infected cell) (__cell-cell interaction__). *vs can NOT recognize or directly interact with free soluble Ags in PBC or extracellular fluids, or Ags on MOs.	__MHC molecules__ clefts bind short linear Ag peptides ONLY (but NO other chemical structures) & to display on host cell surface in structurally stable heterotrimer form.
5- Different TLs have to respond to microbial Ags in different __cellular compartments__. *vs TCRs for Ags can NOT distinguish b/w extracellular & intracellular MOs.	*CD4 & CD8 co-receptors bind to non-polymorphic regions of class II & class I MHC molecules → CD4+ & CD8+ TLs __preferentially recognize__ Ags sampled from extracellular & intracellular (cytosolic) compartments respectively. *__Peptide segregation__ & __Physiologic significance of MHC-associated Ag presentation__: → optimal IRs: -free __extracellular__: CD4+ HTLs, BLs, Abs. -__intracellular__ (inaccessible to Abs): CD8+ CTLs kill infected cells & eliminate reservoir of Inf.

Protein Ags capture & functions of APCs

1- APCs = specialized cells w can capture, transport, present, & display ptn Ags to $CD4^+$ HTLs.

2- All nucleated cells can display ptn Ags to $CD8^+$ CTLs, (however they are NOT called APCs).

General properties of APCs for activation of $CD4^+$ HTLs

1- Professional APCs (DCs, macrophages, & BLs):

(a) **DCs**:

*The ONLY DEDICATED APCs.

*The ONLY & MOST EFFICIENT for activation of **naive** HTLs.

(b) **Macrophages & BLs**: mostly for activation of **effector (**previously activated, & differentiated by previous Ag stimulation, & primary TCM-IR) TLs.

2- APCs functions:

(a) display **Ag peptide-class II MHC complexes** for recognition by HTLs (**First signal**).

(b) express **Costimulators** (membrane-bound ptn molecules, more imp for activation of naive than effector & memory HTLs) → (**Second signal**) for activation of HTLs.

(c) secrete **Cytokines** → HTLs activation.

3- APCs' functions are enhanced:

- **Naturally**: by exposure to **microbial products** (IS responds to MOs better than to harmless, non-microbial substances).

*Activated APCs express: (1) **TLRs & Other Microbial Sensors (OMSs)**, & (2) **Chemokine receptor** (CCR7) → co-localization (maximum chance).

- **Artificially (in immunization/ vaccination)**: by addition of **Adjuvants** (either products of MOs, as killed mycobacteria, or mimic MOs) to purified soluble ptn Ags → induction of $CD4^+$ TCM-IRs.

4- Bidirectional interaction: APCs displaying Ags to HTLs receive signals from HTLs recognizing these Ags w activate APCs & enhance APCs functions: How?

- **Role of CD40 pathway (↑ both TL activation & IR)**: Activated CD4+ HTLs → ↑ **CD40L** → + CD40 → ↑ B7 (***Positive Amplification feedback loop)** & indirectly activates/ licenses **(*Licensing phenomenon)** DCs to become (better, more potent/ efficient) **activated** APCs, → ↑ B7 & cytokines as IL-12 & IFN-γ.

Dendritic Cells (DCs)

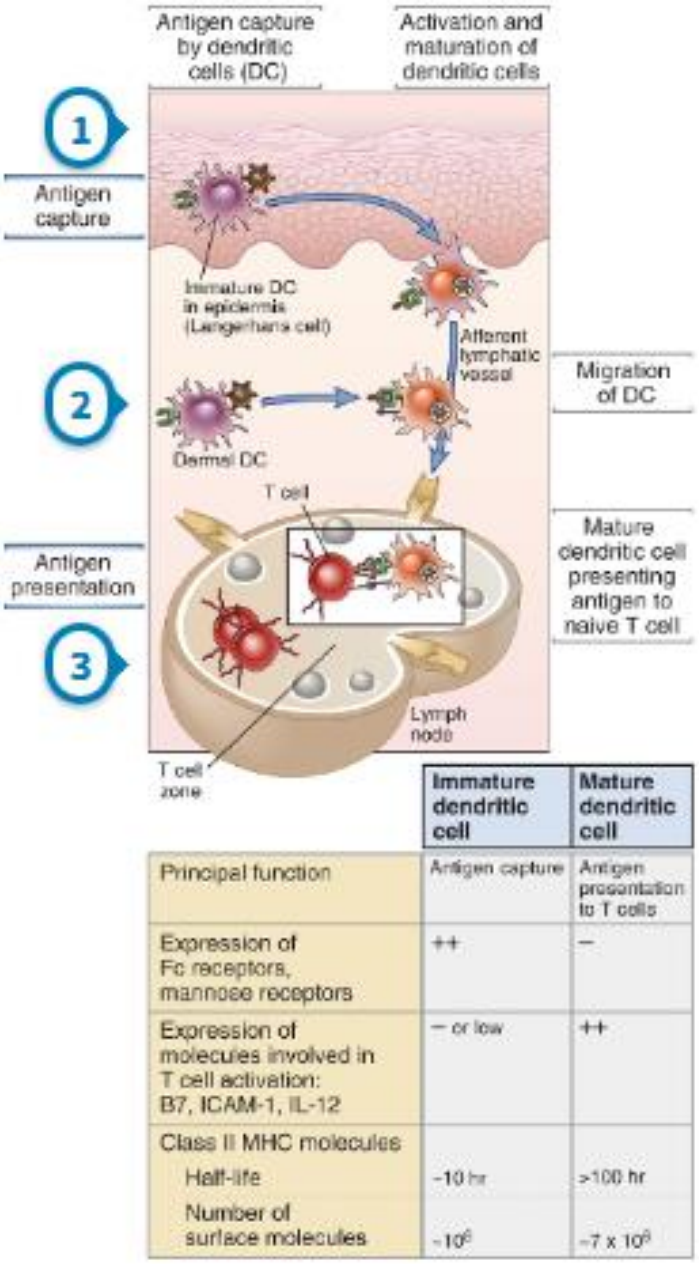
- BM-derived/ **myeloid** lineage; develop (& monocytes) from common precursor cell.
- extensive long spine-like cytoplasmic membrane projections to capture microbial Ags.

Two main types of DCs

- 1- **Conventional (Myeloid)**
- most numerous in SPLOs.
- → DCs resident in epithelia & tissues: (1) Langerhans cells in skin epidermis, (2) Dermal DCs in skin dermis, & (3) Interstitial DCs in most other parenchymal tissues.
- after activation by MOs or cytokines, they mature & migrate to draining LNs.
- Cytokines: TNF & IL-6
- Function: induction of TCM-IRs against most Ags.

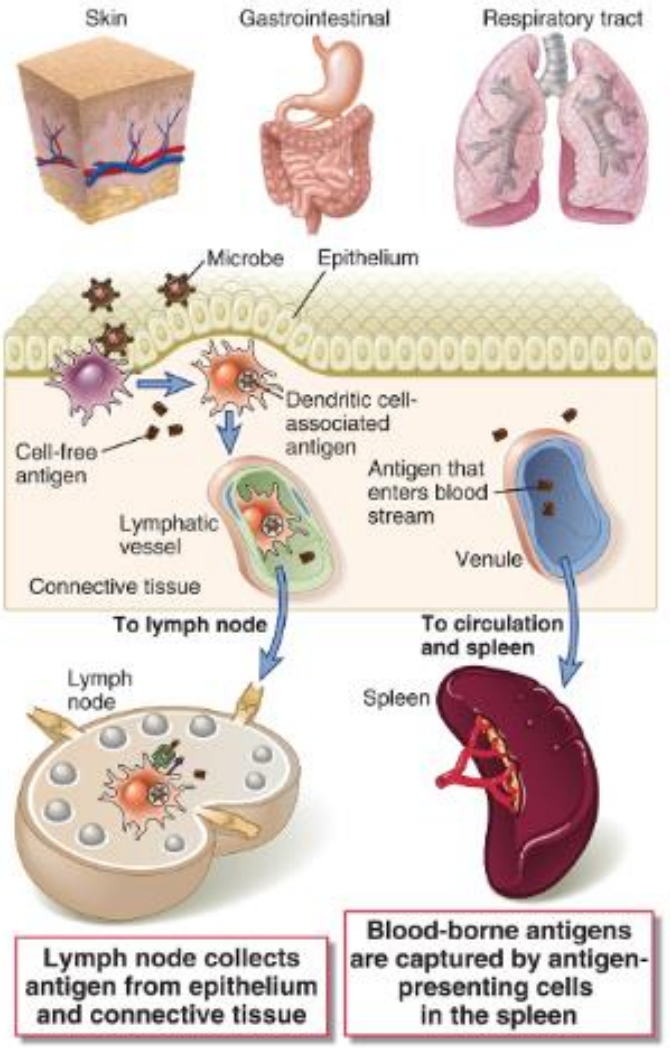
- 2- **Plasmacytoid** (resemble plasma cells morphologically)
- small #s esp in TL zones of spleen & LNs.
- after activation, they acquire morphology & functional properties of DCs.
- Cytokines: Type I IFNs
- Function: innate immunity & induction of TCM-IRs against viruses.

DCs
Immature: (resting, resident in tissue) *in absence of Inf/ inflammation: ***able to capture ptn Ags** (w *cross epithelial barriers, *produced in parenchymal tissues, or *enter PBC) → **Internalization**: (1) C-type lectins receptor-mediated Cytosis (endo- & phago-) of MOs & their Ags (2) without specific recognition receptors Pinocytosis (micro- <0.2µm & macro- 0.2-10µm in diameter) of soluble Ags.. ***but unable to activate TLs.** *may present SELF Ags to SRTLs → inactivation or death, or generation of Tregs → maintain self-tolerance & prevent autoimmunity.
Activation: by exposure to *MOs & microbial products released D innate IR & recognized by TLRs & OMSs in DCs & other cells, & *cytokines (as TNF).
Migration/ Transport to draining regional LNs: *Activated DCs: *lose their adhesiveness for epithelia or tissues. *express (as naïve TLs) CCR7 w bind its specific 2 CCL (19 & 21) produced in same T cell zones of LNs → **Co-localization** (Maximum Chance).
Maturation: Mature (resident in LNs) DCs become **Efficient** APCs (express ↑Ag peptide-MHC complexes, costimulators, & cytokines → naive TLs activation).



	Immature dendritic cell	Mature dendritic cell
Principal function	Antigen capture	Antigen presentation to T cells
Expression of Fc receptors, mannose receptors	++	–
Expression of molecules involved in T cell activation: B7, ICAM-1, IL-12	– or low	++
Class II MHC molecules		
Half-life	~10 hr	>100 hr
Number of surface molecules	~10^6	~7×10^6

- **<u>Strategic anatomic location of DCs</u>**: in *SPLOs, *common portals of microbial entry (skin & mucosal epithelia of GI & RTs), & *sites of foreign microbial protein Ags production (any colonized or infected parenchymal tissue/ organ).
- **<u>Role of DCs in Ag capture</u>**:
- all cell-associated microbial Ags captured by DCs → LVs → Ags concentrated in regional LNs.
- ↓MW microbes & free soluble **<u>ptn</u>** Ags in lymph → LVs independently of DCs → LNs → captured by Resident DCs (& macrophages & BLs) in LNs.
- Soluble inflammatory mediators, as chemokines (produced at sites of Inf) → LVs.



Collection & concentration of foreign Ags in LNs are supplemented by 2 other anatomic adaptations w serve similar functions

- Peyer's patches of ileum & pharyngeal tonsils (= specialized collections of secondary lymphoid tissues in **GIT & RT** mucosa) directly sample luminal contents for foreign Ags.
- Resident APCs in **spleen** sample & capture soluble ptn Ags entering PBC either *directly from tissues or *indirectly by lymph from thoracic duct.

Functions of other cell types as APCs in different situations

- **Macrophages** present Ags of phagocytosed MOs → $CD4^+$ HTLs activate macrophages to kill MOs (CM-IRs vs DTH).
- **BLs** process ingested protein Ags → HTL-dependent Ab IRs.
- **All nucleated cells** process cytosolic protein Ags (viral & tumor Ags, & phagocytosed MOs or their Ags escaping from phagocytic vesicles into cytosol/ cross presentation) → $CD8^+$ CTLs eliminate these APCs.
- **Vascular endothelial cells in humans** express class II MHC molecules & may present Ags to blood TLs adherent to BV wall → recruitment & activation of effector TLs in CM-IRs. **Endothelial cells in grafts** are targets of TLs reacting against graft Ags. **Thymic epithelial cells** constitutively express MHC molecules & present peptide-MHC complexes to maturing TLs in thymus as part of selection processes w shape repertoire of TL specificities.

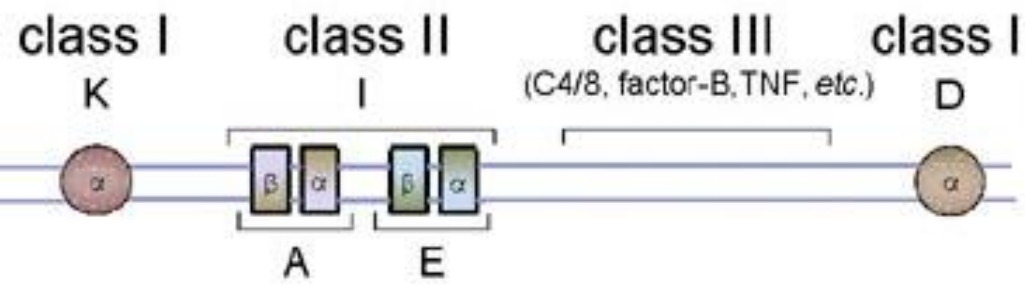
Definitions

- **Human Leukocyte Ags (HLA)**: human MHC ptn molecules/ Ags (first detected on human WBCs & first Ided to cause very strong TCM-IR & as most imp in rejection of transplanted foreign tissue grafts b/w 2 genetically different individuals).
- **H-2 Ags**: mouse MHC Ags.
- Mouse H-2 proteins & HLA proteins have essentially identical structure.

Mouse MHC genes (located on chromosome 17)

Class I

- consists of 2 major loci: K & D.
- Unlike human, loci are NOT together but separated by class II & class III genes.



Class II

- contains 2 loci (A & E), each of w code for 1 alpha & 1 beta chain polypeptide, w form 1 class II molecule.
- known as (I region) & genes in this complex are referred to as [Ir (immune response) genes] since they determine magnitude of IR of different mouse strains to certain Ags.

Products of A & E loci are termed IA & IE Ags, collectively known as Ia Ags.

Human MHC genes (located on chromosome 6)

- MHC locus (large genetic region), contains 3 major classes of genes w encode MHC molecules:

1- Class I & class II: specialized plasma membrane ptn Ags expressed on cell surface.

2- Class III: have NO role in graft rejection & represented on proteins in serum & other body fluids:

*Complement proteins: C4, C2, & factor B.

*Cytokines: TNF.

*HLA-DM.

*Proteasome & TAP.

- **MHC-encoded** (encoded by gene in MHC locus) vs **Non-MHC-encoded** (encoded by gene outside MHC locus).

MHC Molecules Inheritance

- **Haplotype:** group of genes on single chromosome
- **MHC haplotype** = set of MHC alleles present on each single chromosome.
- MHC genes are inherited as haplotypes → each heterozygous individual has 2 haplotypes, 1 from each parent (1 paternal & 1 maternal).
- Each heterozygous individual will have only few different MHC molecules (6 class I & >10-20 class II) on every cell.

Expression of MHC molecules on cells

- MHC genes are NOT somatically rearranged, but rather allelic variations are encoded in germline & are inherited.
- MHC genes are expressed in **co-dominant** manner:

*For any MHC gene, each **individual** has 2 inherited alleles, 1 from each parent → products of both parental genes are found on same cells → maximizes # of MHC molecules for this individual.

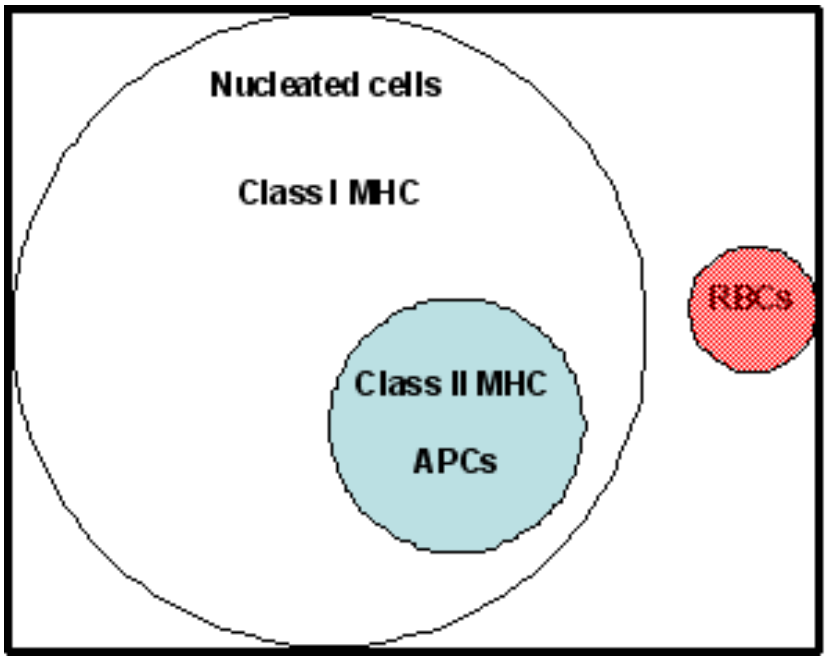
*Each gene has many different alleles in **population** → very rare for 2 individuals to have identical HLA alleles, unless they are identical twins.

Expression of MHC molecules on cells

- **Constitutive expression**: (NOT ALL cells express both class I & class II Ags):

1- Class I Ags on all nucleated cells & platelets (& RBCs in mouse).

2- Class II Ags selectively/ only on APCs as DCs, macrophages, BLs, & few other cell types inc vascular endothelial cells & thymic epithelial cells.



Expression of MHC Molecules on cells

- **Increased by cytokines** produced D both innate & adaptive IRs:

*IFN-α, β & γ (produced D early innate IR to many viruses) → ↑ **class I** on most virally-infected cells (one mechanism by w innate IRs stimulates adaptive IRs).

*IFN-γ (produced by NK cells D innate IRs, & by Ag-activated TLs D adaptive IRs) = principal cytokine → ↑ **class II** on APCs (as DCs & macrophages) → ↑ response to signals from TLRs responding to microbial components → ↑ display of microbial Ags.

*Ag recognition & cytokines produced by HTLs → ↑ **class II** in BLs & Ag presentation to HTLs.

Expression of MHC Molecules on cells

- **Rate of transcription is the major determinant of level of MHC molecule synthesis & expression on cell surface:**

*Cytokines stimulate transcription of class I & class II genes in different cell types.

*IFN-γ stimulates synthesis of **class II transcription activator (CIITA) ptn** = master regulator of class II gene expression).

*Mutations in transcription factors → defective expression of MHC molecules → human immunodeficiency dizs (e.g., bare lymphocyte syndrome).

***Knockout mice** lacking CIITA → ↓ or absent class II expression on DCs & B cells, & inability of IFN-γ to induce class II on all cell types.

Common structural cccs of all MHC Molecules

1- Each MHC molecule consists of:

- **Extracellular** (outside plasma membrane) segment:

***Peptide-binding cleft/ groove** (two parallel α-helical sides/ walls resting on eight-stranded antiparallel β-pleated sheet floor?): formed by interaction of **amino (N)-terminals** of MHC-encoded ptn chains.

***Non-polymorphic Ig-like regions** of class I & class II molecules w contain binding sites for TL co-receptors CD8 & CD4 respectively.

- **At carboxyl (C)-terminal(s) of chain(s)**:

***Transmembrane** short segment(s) of hydrophobic AA residues traversing lipid bilayer of plasma membrane → (anchor) in cell membrane.

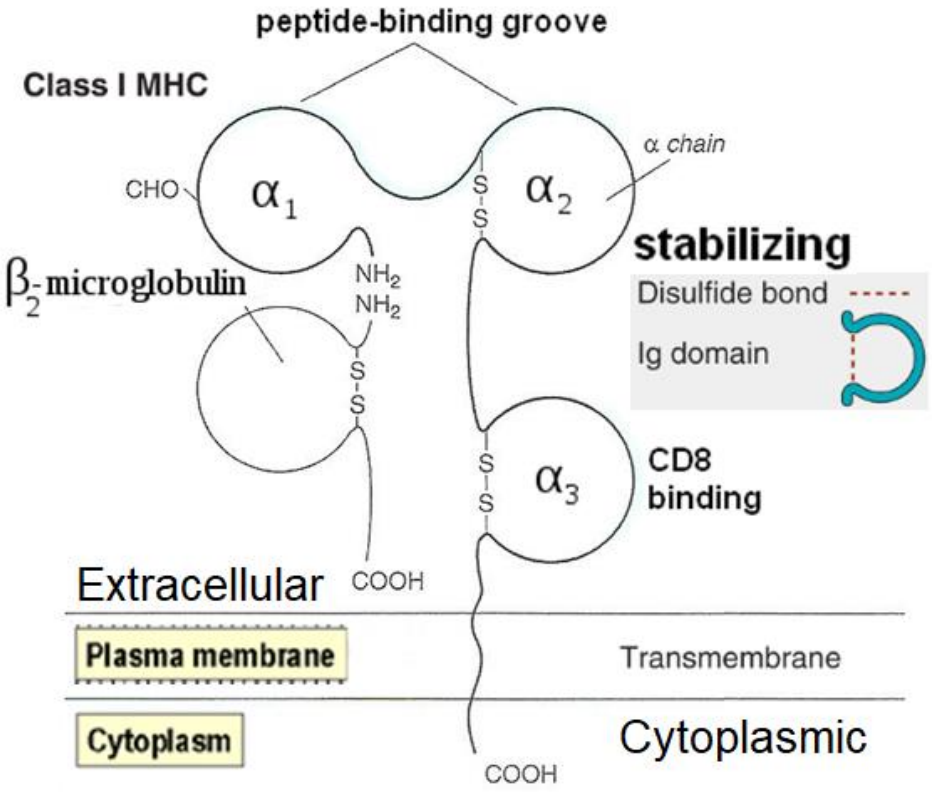
***Cytoplasmic** short segment(s) of basic hydrophilic AA residues containing sites for phosphorylation & binding to cytoskeletal elements.

2- **MHC polymorphism (polymorphic AA residues)**: located (concentrated) in cleft.

- MHC genes are the most polymorphic of any in the human genome.
- Both class I & class II MHC genes are polymorphic, however class I MHC genes are more polymorphic.
- **Polymorphic** (vary among different MHC alleles of particular gene → most individuals are heterozygous for MHC genes) vs **Non-polymorphic** (invariant/ do NOT vary among different MHC alleles of particular gene).

Structure of class I MHC molecule

- **Protein dimers**: [Homodimers (complexes of identical monomers) vs Heterodimers (complexes of non-identical monomers)].
- Heterodimer of 2 non-covalently attached polypeptide chains (=α heavy chain + β_2-microglobulin light chain).



- **α (Heavy) chain**:

*MHC-encoded, polymorphic & glycosylated.

*44-47 kD, long.

*α3 segment is folded into Ig domain & contains binding site for CD8.

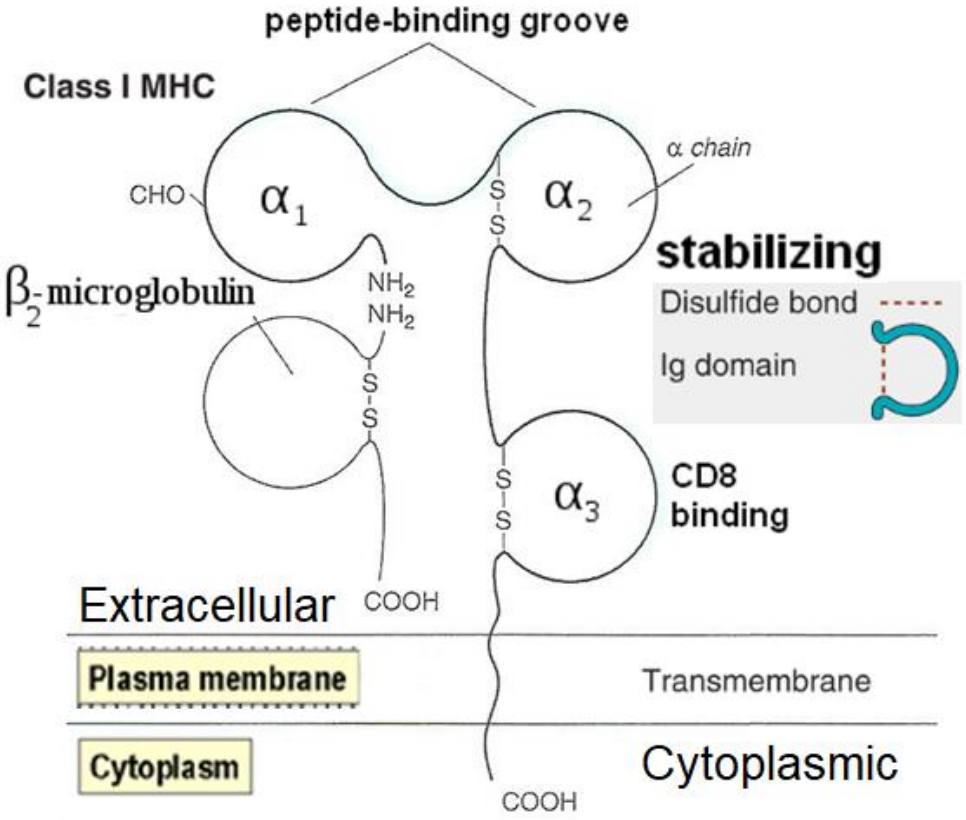
- **β_2-Microglobulin (β_2m, Light chain)**:

*Named acc/to: electrophoretic mobility (β_2), size (micro; 12-kD, short NOT anchored in membrane) & solubility (globulin).

*Non-MHC-encoded, Non-polymorphic & Non-glycosylated.

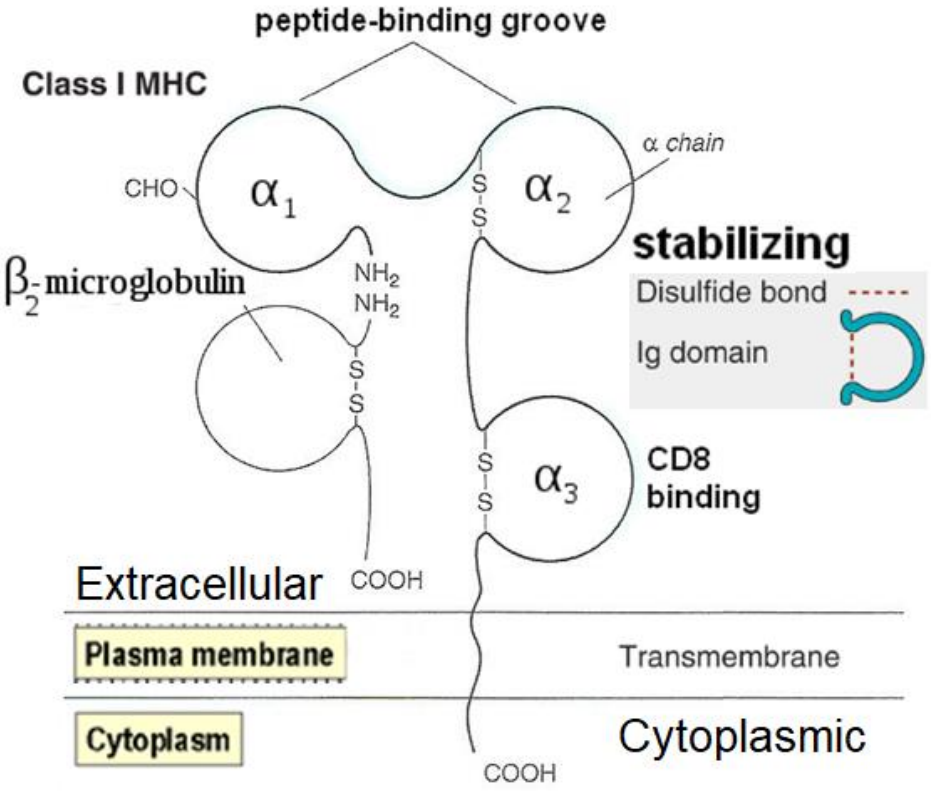
*Folded into Ig domain (similar to α3 segment).

*Closely ass/with α3 domain → maintain proper conformation/ shape of MHC molecule.



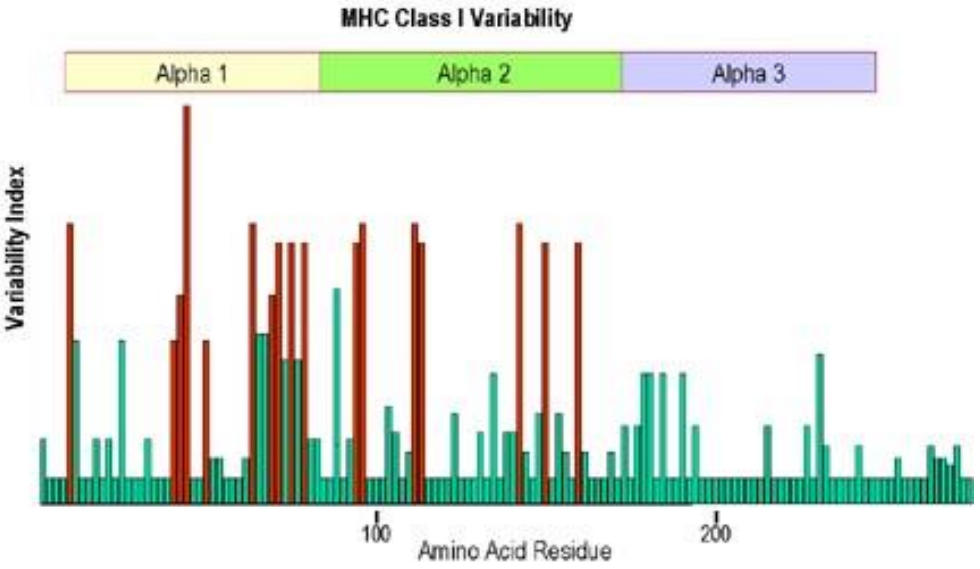
Peptide-binding cleft

- Formed by interaction of amino (N)-terminals of α1 & α2 segments of α chain.
- Ends are **CLOSED** → bind smaller peptides 8-11 AAs long residues → ends of peptide are buried within closed ends of cleft while center bulges out for presentation to TCR.
- Dimer is structurally unstable.
- Fully assembled **trimer** including Ag peptide is structurally stable → only potentially useful Ag peptide-loaded MHC molecules are expressed on cell surfaces.



Polymorphic residues

- Most polymorphic highly variable AAs residues located at different positions along α chain are most pronounced in α1 & α2 segments w line floor & wall of cleft, make contact with peptide & contribute to variations among different class I alleles in peptide binding & TL recognition.



Class I MHC gene

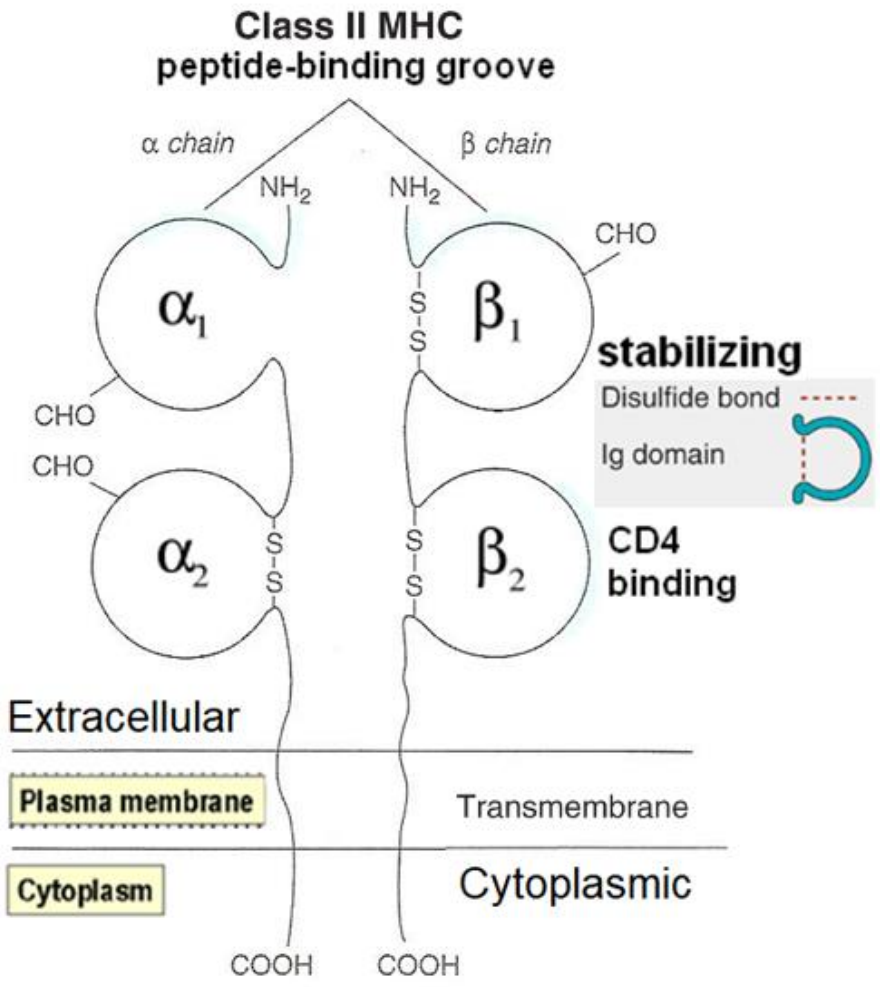
- There are 6 loci (each has gene for α chain w is encoded by 2 inherited alleles) → 6 different class I molecules on every cell.
- Loci are designated as: HLA-A, HLA-B, HLA-C, HLA-E, HLA-F & HLA-G.
- HLA-A, HLA-B & HLA-C are most imp & most polymorphic.

- Individuals with defective beta-2 microglobulin gene do NOT express any class I molecule on cell surface → deficiency of CTLs.

Polymorphism of class I MHC genes	
Locus	**Number of alleles (allotypes)**
HLA-A	218
HLA-B	439
HLA-C	96
HLA-E, HLA-F and HLA-G	Relatively few alleles

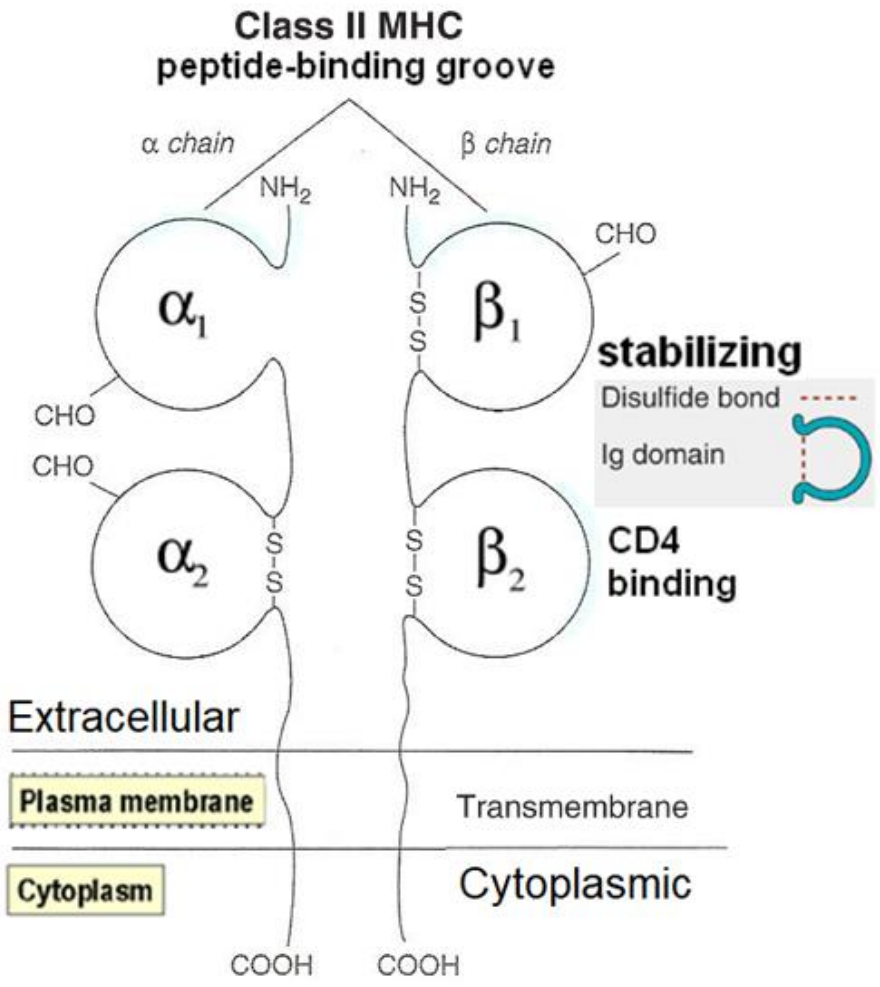
Structure of class II MHC molecule

- Heterodimer of 2 non-identical non-covalently attached polypeptide chains: α (32-34-kD) & β (29-32-kD).
- Both are MHC-encoded, polymorphic & glycosylated.
- α2 & β2 segments are folded into Ig domains & are non-polymorphic.
- β2 segment contains binding site for CD4.



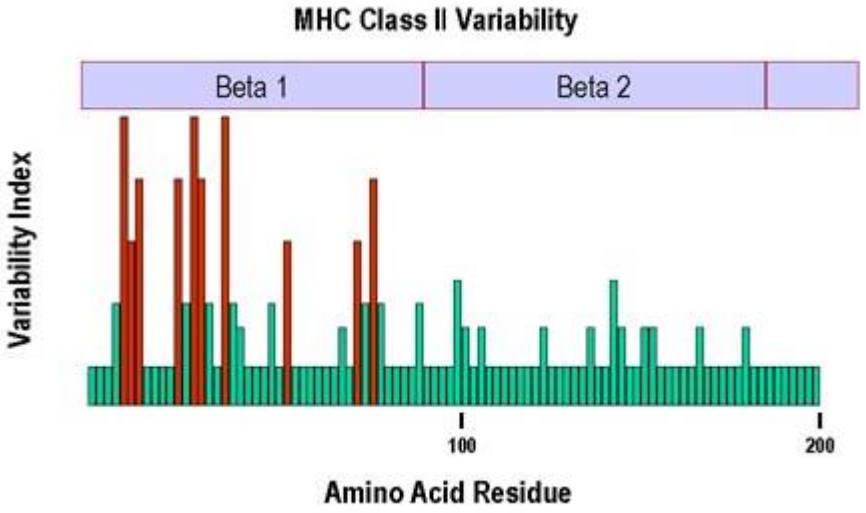
Peptide-binding cleft

- Formed by interaction of amino (N)-terminals of α1 & β1 segments of the 2 chains (four strands of floor + one α-helical wall formed by α1 segment, & other four strands of floor & second α-helical wall formed by β1 segment).
- Ends are **OPEN** → bind longer peptides 10 - ≥30 AAs long residues w extend at either end beyond floor of cleft.
- Dimer is structurally unstable.
- Fully assembled **trimer** including Ag peptide is structurally stable → only potentially useful Ag peptide-loaded MHC molecules are expressed on cell surfaces.



Polymorphic residues

- Most polymorphic highly variable AAs residues are located in α1 & β1 segments w line floor & wall of cleft, make contact with peptide & contribute to variations among different class II alleles in peptide binding & TL recognition.
- Greatest polymorphism for β chain is in β1 segment.



Class II MHC gene

- There are 5 loci (each has 1 gene for α chain & at least 1 gene for β chain, each gene is encoded by 2 inherited alleles) → >10-20 different class II MHC molecules on every cell.
- Loci are designated as: HLA-DP, HLA-DQ, HLA-DR, HLA-DM & HLA-DO.
- Among these, HLA-DP, HLA-DQ & HLA-DR are most imp & most polymorphic.
- DR locus may contain >1, possibly 4, functional beta-chain genes.

Polymorphism of class II MHC genes

Locus	Number of alleles (allotypes)
HLA-DPA	12
HLA-DPB	88
HLA-DQA	17
HLA-DQB	42
HLA-DRA	2
HLA-DRB1	269
HLA-DRB3	30
HLA-DRB4	7
HLA-DRB5	12
HLA-DM and HLA-DO	Relatively few alleles

Structural Basis of Peptide Binding to MHC Molecules

- **Non-covalent binding** : *through electrostatic forces, hydrogen bonds, & van der Waals interactions, & *b/w AAs residues in both MHC molecules clefts (positively charged N terminus) & Ag peptides (negatively charged C terminus).
- Peptides bind to MHC clefts in **extended conformation/ shape**.
- Once bound, peptides & their associated water molecules fill clefts → **extensive contacts** with AAs residues forming β strands of floor & α helices of walls of MHC cleft.
- Affinity of peptide-MHC interactions is NOT altered by chemokines.

CCCs of Ag peptide-MHC binding

1- Each MHC molecule has:

***SINGLE** cleft → binds only one peptide at a time.

***BROAD** specificity → binds large #s of different peptides w share common structural features (e.g., size, & Anchor residues).

2- Binding interaction:

***Occurs** D biosynthesis & assembly of MHC molecules inside cells & before cell surface expression .

***Stable & saturable** (→ long half-lives of hours-many days) with extraordinary **very slow off-rate of peptide dissociation** (→ long enough peptide display → Maximum Chance.

APCs continuously present on their surface, peptides derived from all proteins they encounter & only very small fraction of cell surface peptide-MHC complexes will contain same peptide:

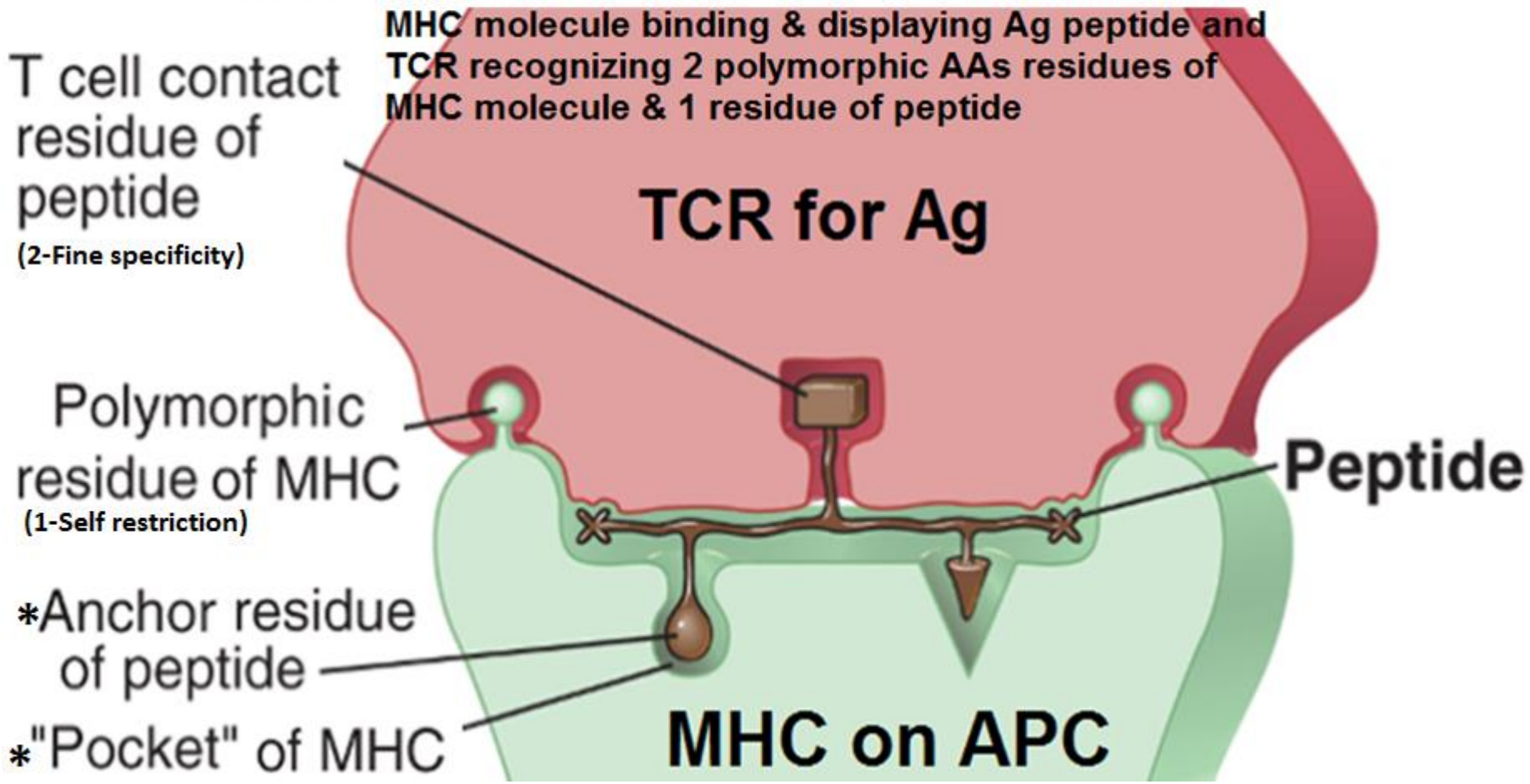
3- **Very small #s** of Ag peptide-MHC complexes are required to activate specific naïve TLs (e.g., ≈100 complexes of Ag peptide & class II MHC molecule = <0.1% of total # of class II MHC molecules on typical APC's cell surface).

4- MHC molecules display both & are unable to discriminate b/w:

***Non-self foreign peptides** (derived from foreign microbial ptns): TLs are remarkably sensitive & specifically recognize any foreign Ag peptide-MHC complexes to be activated) &

***Self peptides** (mostly normally displayed & derived from self ptns): so NOT to induce autoimmunity, TLs cannot normally respond to self Ags & TLs specific for self Ag peptide-MHC complexes are killed or inactivated).

Highly polymorphic AAs residues	Peptide-binding-cleft of MHC molecule	Bound Ag peptide
MHC peptide-binding specificity	**Pockets** structures	Complementary **Anchor residues** (usually only 1-2 to allow greater variability in other AAs residues of peptide, w are recognized by specific TLs)
TCR Ag peptide recognition specificity	1- Only self (MHC restriction)	2- Fine specificity



MHC restriction of TLs, MHC-restricted Ag recognition

- Normally, TLs specifically recognize ONLY **foreign** protein Ag peptides, bound to host **self**-MHC protein molecules, & expressed on surfaces of other cells. (→ self-nonself discrimination).

Immunogenicity of ptn Ags determination by MHC molecules

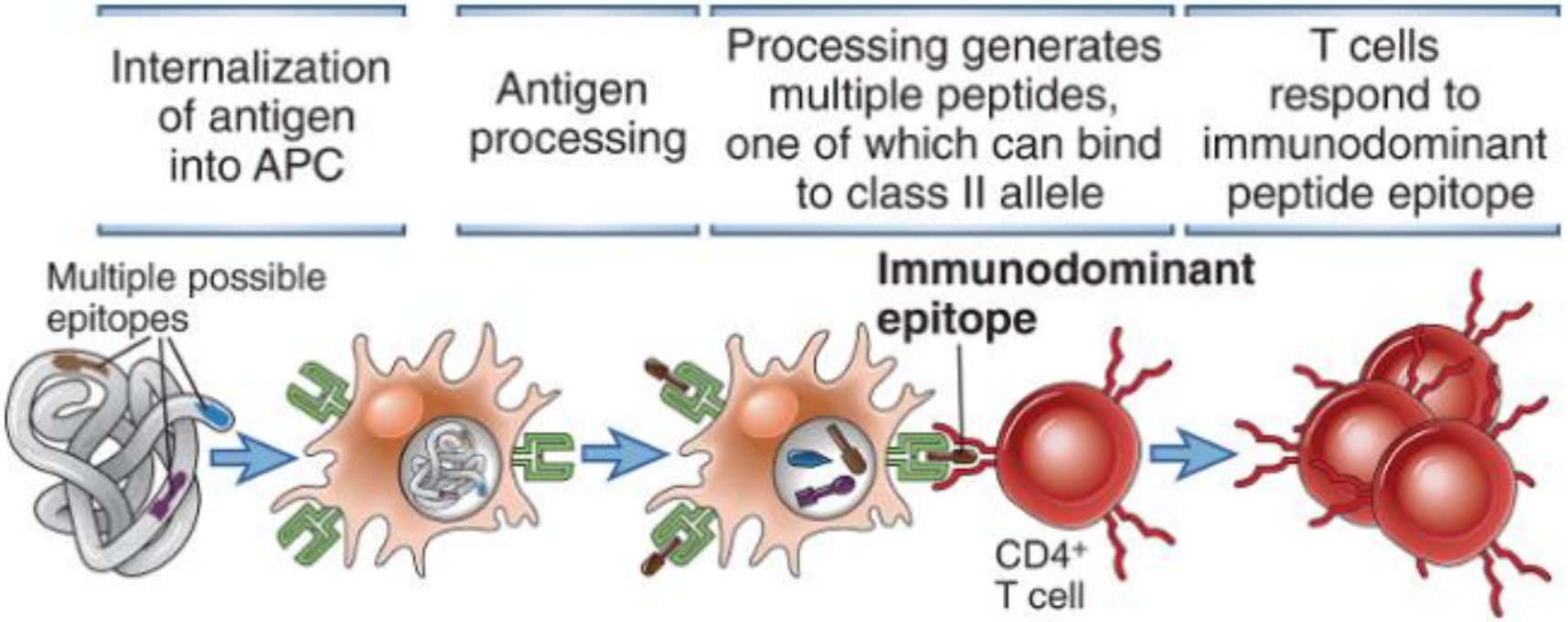
1- Processing of ptn Ags → multiple peptides; only one or few are immunodominant with **Immunodominant determinants or epitopes** (linear AA sequences) to w majority of responding TLs are specific, & w bind to available class I & class II MHC molecules with high affinity & elicit strongest specific TCM-IRs.

*Application: vaccines' design.

- 2- **Immune response (Ir) genes** (structural basis for function of MHC genes as Ir genes) = class II MHC genes w determine IRs to particular Ags by expression of different MHC alleles in different individuals to favor binding/ displaying of different Ag peptides → specific HTLs activation.

Immunodominance of peptides

(Fig: extracellular Ag generating class II-binding peptide, but this also applies to peptides of cytosolic Ag w are presented by class I MHC molecules)



Conventional MHC pathways of Protein Ag presentation		
Pathway	Class I (Cytosolic, Endogenous)	Class II (Vesicular, Exogenous)
self & foreign (microbial) **protein Ags**, their cellular locations & site of processing & synthesis of Ag peptides →	Cytosolic (intracellular, mostly endogenously synthesized in cell cytoplasm (cytosol compartment) as viral & tumor proteins	Vesicular (extracellular, mostly exogenously environmental & internalized within vesicles (endosomes/ lysozomes)
bound non-covalently to/ displayed by/ presented by/ expressed in ass/w----- **MHC molecules** (expression) →	class I (on All nucleated cells, platelets, NOT RBCs)	class II (Only on APCs as DCs, macrophages, BLs & few other cell types inc endothelial cells & thymic epithelial cells).
for Ag peptide specific recognition by TCR on & selective stimulation/ activation of ------- →	class I MHC self -restricted CD8+ CTLs	class II MHC self-restricted CD4+ HTLs *Naive: DCs. *Differentiated: Others.
most effective TCM-IRs (effector function) for eliminating that type of MOs.	Eliminate intracellular Ags -Kill (lyse) all nucleated cells w express Ags: *viral, *tumor.	Eliminate extracellular Ags: -BLs (Humoral/ Abs). -Macrophages (Cellular/ intracellular killing).

Processing of protein Ags

- **MHC molecules** are synthesized & assembled in ER & can bind & display ONLY linear Ag peptides w are generated in different cellular locations & transported to ER for binding with MHC clefts & forming structurally stable trimers to be expressed on cell surfaces.

Class I MHC pathway for processing & presentation of cytosolic protein Ags

1- **Sources**:

*most are synthesized within cells: as *viruses or other intracellular MOs, & *mutated or overexpressed genes in tumor cells.

*some (as microbial & other particulate protein Ags) are phagocytosed, processed, & transported into cytosol (Cross-presentation).

2- **Processing** (Major mechanism):

*Tagging by covalent binding with **ubiquitin** (= small polypeptide) → activation of proteolysis by **proteasome** (multiprotein proteolytic E, found in cytoplasm & nuclei of most cells).

*IFN-γ enhances Ag presentation: *change in substrate specificity of proteasome to produce C termini typical of Ag peptides transported into class I pathway. *↑ expression of MHC molecules.

3- **Transport of generated peptides into ER**

*by active, ATP-dependent pump **T**ransporter **A**ssociated with Ag **P**rocessing (**TAP**; heterodimer ptn located in ER membrane).

*On luminal side of ER membrane, **TAPASIN** (ptn with affinity for & brings TAP into complex with newly synthesized nascent class I dimer).

4- **Assembly of trimers in ER**

*Folding & assembly of structurally unstable dimer are aided by ER-resident **chaperone** ptns (e.g., membrane chaperone **calnexin**, & luminal chaperone **calreticulin**). (Tapasin, calnexin & calreticulin regulate assembly).

***Trimming**: of Ag peptides by **ER-associated peptidase (ERAP)** to appropriate size for MHC cleft → release of structurally stable trimer → exit from ER to Golgi complex → transport by **exocytic vesicles** to cell surface → 5- **Expression**.

Class II MHC pathway for processing & presentation of vesicular protein Ags

1- **Generation**:

- Most are captured from extracellular environment & **internalized** into endosomes by specialized APCs:

*DCs & macrophages express different surface receptors w recognize & bind common structures shared by many MOs.

*Macrophages express receptors for both (Fc of Abs, & complement protein C3b), w bind coated Ags.

*BLs express specific surface Ig receptor with high affinity for protein Ags present at very [low]s in extracellular fluid.

- **Internalization into endocytic vesicles**:

*Protein Ags → **endosomes** (intracellular membrane-bound vesicles).

*Particulate MOs → phagosomes w fuse with lysosomes (more dense membrane-bound E-containing vesicles) → phagolysosomes or secondary/ **late lysosomes**).

2- **Processing**: by active process mediated by **proteases (cathepsins)** (proteolytic Es w function at acidic pH).

3- Biosynthesis & transport of class II MHC molecules:

*Folding & assembly of structurally unstable newly synthesized nascent class II dimers are aided by ER-resident chaperone ptns as **calnexin**.

*Transport to endosomes ass/with **invariant chain**; (I_i, trimer ptn) w occupies & blocks clefts.

Reminder: in ER, transported peptides preferentially bind to class I but NOT class II MHC molecules, Why?

(1) New class I dimers are attached to luminal aspect of TAP complex & they capture peptides rapidly D their transport into ER by TAP.

(2) clefts of new class II dimers are blocked by I_i.

- **MHC class II compartment (MIIC)** = subset of late endosomes in macrophages & human BLs w contains *proteolytic Es (proteases; cathepsins), *class II MHC-molecules, *invariant chain, & *HLA-DM molecules.
- New class II dimers ass/with I_i transported from ER to endosomal vesicles → I_i degraded by proteolytic Es (proteases; cathepsins) → **class II-associated invariant chain peptide (CLIP)** (small 24-AA peptide remnant of I_i) → **HLA-DM molecules** (MHC-encoded, structurally similar to class II MHC molecules but differ in being NOT polymorphic & NOT expressed on cell surface, act as peptide exchanger; facilitating removal of CLIP from cleft & accelerating addition of peptides to class II dimers).

4- Association of processed Ag peptides with Class II MHC molecules in vesicles:

- D transport toward cell surface, **exocytic vesicles** containing class II molecules meet & fuse with **endocytic vesicles** containing peptides → **Trimming**: of typically large Ag peptides w bind open-ended clefts by proteolytic Es to appropriate size for MHC clefts → structurally stable trimers in endocytic vesicles → release, delivery & display on cell surface of APC → **5- Expression**

Cross presentation or cross-priming

- Violation of (conventional pathway of Ag presentation) rule.
- **Mechanism**: D fusion of vesicles (endosomes/ phagosomes) containing initially internalized protein Ags with ER → transport of Ags to cytosol → conventional class I cytosolic pathway of Ag presentation.
- = one cell type (DCs) can present Ags from another cell.
- Ags are produced in virus-infected or tumor cells w are incapable of presenting Ag.
- DCs capture & ingest these Ag-containing cells w can be internalized into vesicles, present endocytosed vesicular viral or tumor Ags in ass/with class I MHC molecules for recognition by naive CD8+ CTLs → prime/ activate naive CD8+ CTLs specific for these Ags. (NB. At same time, same cross-presenting DCs can display peptides generated in vesicles in ass/with class II MHC molecules for recognition by $CD4^+$ HTLs, w are often required to induce full responses of $CD8^+$ cells).

Functions of other TL subsets

- Exception to rule: (TLs can see only MHC-ass Ag peptides).
- NKT & γδ-TLs (TLs with γδ TCR) are smaller populations of TLs with common cccs w distinguish them from majority of TLs (CD4+ HTLs & CD8+ CTLs):

1- recognize wide variety of Ags inc peptides, however many are non-ptn Ags as lipids (NKT cells) & small molecules (γδ-TLs) without processing or involvement of class I or class II MHC molecules (**MHC-independent, Not MHC-restricted)**.

2- express Ag receptors with limited diversity w recognize invariant & conserved microbial Ags (ligands) & respond against small group of MOs.

3- abundant in epithelial tissues, as GIT.

NKT cells

- express markers w are ccc of both NK (as CD56) & TLs.
- recognize lipids & glycolipids Ags displayed by **CD1** (ptn molecules, Non-MHC encoded, nonpolymorphic, “non-classical” class I MHC-like; structurally homologus to class I MHC alpha-chain, associates with beta2-microglobulin).
- may mediate protective innate IRs against some mycobacteria with lipid-rich cell walls.

γδ-TLs

- <5% of all TLs vs more numerous TLs with αβ-TLs (TLs with αβ TCR).
- recognize small phosphorylated molecules & alkyl amines.

Chemicals

- Some TLs are specific for small chemical **haptens** (as *dinitrophenol, *urushiol of poison ivy, & *β lactams of penicillin Abx) w covalently bind to self ptns → hapten-conjugated peptides with novel peptide determinants w are recognized by these TLs.
- Contact-sensitizing chemicals (introduced through skin) are presented to CD4+ or CD8+ TLs → TL reactions (**contact sensitivity reactions**).

THANK YOU