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ANTIGEN PROCESSING & PRESENTATION

Intro -

* B-cell receptors / Abs can recognise Antigens alone.
* T-cell receptors can recognise antigenic peptides that are only Presented by APC via MHC.

T cells → T_C (cytotoxic) → $CD8^+$ T-cells → recognises MHC I
T cells → T_H (Helper) → $CD4^+$ T-cells → recognises MHC II

MHC genes -

During grafting, the MHC molecules decide whether the body accepts or rejects the tissue based on self & non-self recognition. However, they have been playing a major role in Antigen recognition & Presentation.

Types:

* Class I MHC → All nucleated cells ; Presents to Tc cells
* Class II MHC → Professional APCs ; Presents to T_H cells.
* Class III MHC

Class I MHC -

→ Made of 2 ~~POLP~~ polypeptide chains — α chain, β_2-microglobulin chain.
→ α chain made of 3 domains : α_1, α_2, α_3, a transmembrane domain & short cytoplasmic tail.
→ β_2 microglobulin do not have transmembrane domain & cytoplasmic tail.
It remains associated with α-chain through non-covalent interaction b/w α_3 domain.
→ The sequence & structure of α_3 domain & β_2-microglobulin is are similar to immunoglobulins, hence they classified under immunoglobulin class.

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→ The α_1 & α_2 domains forms a groove-like structure where the Antigenic peptide will bind & be presented to the T-cells.

→ To a class I MHC molecule, usually about 8-10 AA long Antigenic peptide can bind (smaller peptides bind since the cleft is continuous b/w $\alpha_1 - \alpha_2$ domains).

Class II MHC –

→ Made up of 2 polypeptide chains — α chain, β chain

→ α chain : α_1, α_2 + transmembrane domain + cytoplasmic tail

β chain : β_1, β_2 + transmembrane domain + cytoplasmic tail

→ The antigenic peptide will bind to the cleft b/w the α_1 & β_2 domain.

→ Class II MHC cleft can accomodate bigger Antigenic peptides (13-18 AA long) since it is an open cleft (formed b/w two diff chains)

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NOTE: Liver transplants are more successful due to the fact that the hepatic cells don't express MHC much.

Genetic organization –

MHC I, II ⇒ Chromosome 16

MHC I : α-chain & β-microglobulin comes together in the Endoplasmic reticulum after being coded separately.

MHC II : α-chain & β-chain gets associated in the ER.

There is no genetic recombination involved in MHCs. MHCs. Thats why there is less diversity compared to BCR & TCR.

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However, there is diversity that arises due to polymorphism.
Each MHC can recognise more than one antigenic peptide (8~10).

MHC I → All nucleated cells (but ~~level~~ expression levels vary from cell-to-cell).
→ Presents Host ~~peptides cells~~ peptides (normal protein turn-over) on the surface of healthy cells. Proteins are always produced and degraded → the degraded protein is used for presentation, This presentation helps in the process of thymic selection.
→ Immune cells (the self-binding cells) that binds strongly during thymic selection will undergo apoptosis (negative selection)
→ It can present foreign peptides that's formed from Proteins produced by the pathogens that invaded the cells.
→ Tumour cells also synthesize foreign proteins. That can be ~~recogn~~ presented by MHCs.
→ It processes intracellular proteins / peptides.

MHC II → Processes Extracellular antigens
eg: A virus that's yet to infect a cell
→ Activates Tc cells & TH cells
TH cells activates B cells that produces Abs that help neutralize extracellular antigens.

NOTE: * Professional APCs ~~pre~~ express both MHCs (I & II).
→ Expressed by professional APC (pAPC)
- Dendritic cells → Most effective
- Macro Phages → must be activated
- B-cells → must be activated

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Antigen Processing is necessary for T-cell Activation :-

Experiment:

Model system → Macrophage

Exp(i) – • Macrophages were fixed (freezing cell activity) before exposing it to antigens
↓
using formalin

• Exposed it to antigen for 1 hour

Result: No antigenic peptides present on Macrophage due to ceasing of cellular activity.
No T_H activation observed!

Exp (ii) – • Macrophages were treated unfixed with antigens for 1 hour.
• Cells were fixed.

~~Resi~~

Result: Antigenic peptides were expressed by MHC.
T_H cells were activated!

Exp (iii) – • Macrophages wase fixed first.
• ~~Ex A~~ Exposed it to Antigenic peptides.

Result: MHC ~~ex~~ presented the Antigenic peptides.
T_H cells were activated!

Antigen Processing & Presentation:

Antigens → Exogenous
↘ Endogenous

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(i) Exogenous Antigen
- Produced outside of host cell
- enters cell by Endocytosis or Phagocytosis
- Processed by the Endocytic pathway
- Humoral response is best suited for elimination of exogenous antigens

Endo
- Produced within the host cell
- Processed within the cell, in the cytoplasm, through cytosolic pathway
- Cell-mediated response for elimination of endogenous antigens

OVERVIEW :-

Cytosolic and Endocytic Pathway -

Cytosolic Pathway:
The Endogenous antigens are tagged with ubiquitine and will be degraded by the ubiquitine pathway. The tagged Proteins are carried to the Proteosome complex - where they are degraded into amino acids or some proteins are carried to the endoplasmic reticulum with the help of a transporter Protein called TAP (Transporter of Antigenic peptides). The antigenic peptides will be associated with Class I MHC in the ER itself and will then be carried to the cell surface to be Presented.

Endocytic Pathway:
For exogenous antigens, first the antigens are phagocytosized, then they are send to endocytic compartments (formed by the fusion with Lysozomes). The compartments have Proteases that break the antigens down to smaller peptides which associate with the Class II MHC and are Presented.

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Evidence for two Processing and Presentation Pathways :-

→ Experiment was conducted where all Professional APCs were used since they express both class I and class II MHC

→ They were exposed to different treatments

→ After treatment, cytotoxic T cells were added to determine if the Tc cells can cause lysis of the ~~tar~~ treated cells

→ Determination of MHC class ~~wased~~ was based on the results of cell lysis

→ MHC class used to determine type of antigen presentation

NOTE: Phagocytosis is a non-specific way of engulfing the antigen.

Treatments -

(i) First treatment was with a virus.
Result: Both class I and class II MHCs were presented on the cell surface.

(ii) Second treatment involves using UV infected virus.
Result: Class II MHC was expressed

(iii) Third treatment involves use of Emetine ~~as drug~~ to treat the virus (Emetine is a drug that inhibits viral synthesis)
Result: Only class II MHCs ~~&~~ were expressed.

(iv) Fourth treatment involves treating the virus with Chlorophyll (Chlorophyll inhibits Endocytic pathway by preventing change in pH in Endosome preventing ~~activation of Endo~~ the synthesis of Proteases)
Result: Only class I MHCs were expressed

Conclusion: There are 2 processing pathways for expression of ~~the~~ diff. types of MHCs.

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Cytosolic pathway:

- Processes intracellular antigens
- These proteins will be tagged by ubiquitin & undergoes ubiquitination. } facilitated by E1, E2, E3 enzymes (Ubiquitine molecules)
  (ubiquitin-activating enzyme, ubiquitin-conjugating enzyme, ubiquitin-protein ligases)
- The ~~broken down peptides~~ tagged antigen will enter a complex called Proteosome.
- Proteosome has two cores: (i) Catalytic core (20S) → α, β subunits
  breaks down ubiquitin-tagged antigens into peptides.
  (ii) Regulatory core (19S): Recognise ubiquitin-tagged Proteins and direct to catalytic core
- Proteosome complex is present in the cytoplasm of all the cells of the body.
- In leukocytes → increased expression of proteosome. Some of the β subunits (β1, β2, β5) are replaced by LMP2, LMP10 & LMP7.
  These LMPs are induced in response to IFN-γ (that are produced by activated leukocytes). ↓ control mechanism
- The entry of these peptides into ER is facilitated by a ~~transporter Protein called TAP1 & TAP2 (ATP-dependent).~~ transporter protein called TAP. → ATP-dependent
  TAP is a heterodimer protein (TAP1 & TAP2 association).
- The peptides are assembled into class I MHC inside the ER.
- Class I MHC assembly:
  α chain → synthesized in cytoplasm → enters ER → associates with Calnexin (Chaperone Protein: aids in folding & stability) → gets associated while calnexin gets dissociated ↓ the peptides entering TAP will bind to class I MHC while the two proteins gets dissociated
  & subsequently β2 micro-globulin will be associated with α-chain
  → Tapasin + Calreticulin (Chaperone Protein: aids in folding & stability of MHC I)
  TAP-associated Protein: aids in bringing the class I MHC closer to TAP transporter protein
  → Net Antigenic associated Class I MHC will Exit ER → Move to Golgi complex via vesicles → cell surface

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Endocytic Pathway:

→ Exogenous antigens are processed by Endocytic Pathway where Endosomes are formed.

NOTE: Endocytosis: • Receptor-mediated ~~Phagocytosis~~
• Specific • can ~~b und~~ facilitate lysis

Phagocytosis: • non-specific
• mediates lysis

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→ Endosomes have inactive Proteases.
Upon maturation, pH of Endosomic vesicles decreases that activates the proteases.

→ These proteases will degrade antigens into peptides.

→ Class II MHS assembly:

Class II MHC + Invariant chain, → blocks cleft of MHC II → MHC II – Ic complex moves out of ER via vesicles

Prevents intracellular antigens from binding

~~The~~ ensures class I MHC binding antigens (the intracellular ones) are not binding to MHC II

Upon vesicle maturation, pH changes

CLASS II associated Invariant chain Peptide

MHC II + CLIP ← Cleavage of Ic. ← changes

↳ small peptides (remnants of Ic)

↳ vesicle fusion with Endosome → Release of CLIP → Binding of Extracellular peptide to MHC II cleft via HLA-DM

↳ containing extracellular peptides

↳ only after maturation of peptides in the Endosome & recruitment of HLA-DM

HLA-DO
Prevents activity of HLA-DM
Regulatory Protein
(expressed ubiquitously - ~~ex~~ no need of IFNs)

non-conventional MHC (helps removal of CLIP and binding of extracellular peptide to cleft of MHC II)
(Expressed in Response to ~~interfe~~ interferons)

→ Takes place only in Professional APCs (like Dendritic cells, Macrophages, NKCs).

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cross-presentation:

* Extracellular proteins are processed by the APCs, while intracellular proteins are processed by all nucleated cells.
* However, its the APCs that will activate Adaptive immune response.
* If by chance, the APCs are not ~~a in encoun~~ infected by virus by rather engulf the virus through Endocytosis, cross presentation takes place.
* In cross presentation, Peptides ~~for~~ from Endocytic Pathway sometimes get diverted to cytosolic Pathway where extracellular Peptides gets associated with class I MHC resulting in activation of $CD8^+$ T cells (Tc cells).

Not all Peptides are diverted; some of them are associated with MHC II leading to $CD4^+$ T cells (T_H cells) activation.

NOTE: Cross Presentation is applicable for Dendritic cells ONLY, since they are nucleated cells as well as Professional APC. Making them the most Prominent of the APCs.

Presentation of non-peptide antigens:

Presentation of non-peptide (such as lipid & glycolipid) antigens derived from bacteria involves class I-like CD1 molecules (non-classical pathway).