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Ira Mellman's Lecture Part 2:
Antigen Presentation and Dendritic Cells

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Contents

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1. Review Questions

1. Where in the body can you find immature dendritic cells (DC)? Mature dendritic cells?
2. Both DC and macrophages respond to the same microbial ligands. How do they differ?
3. What is the optimal pH at which lysosomal proteases function? What is the pH of an immature DC's lysosome? Mature?
4. Following synthesis in the ER, where does MHC II go? How does it get there?
5. What lysosomal inhibitor regulates invariant chain cleavage?
6. MHC II is localized to lysosomes in immature DCs, but is found on the cell surface in mature DCs. How is this trafficking regulated?
7. The body produces many different types of T cells, each specialized for a particular type of response. How do DCs direct T cell responses?

8. Which subset of T cells maintains tolerance in adults?

2. Answers to Review Questions

1. Immature DCs reside in the periphery (at mucous membranes, in the skin, etc), while mature DCs have migrated through the lymphatics to secondary lymph organs, such as lymph nodes.

2. Upon TLR binding, macrophages launch a cytotoxic response, while DCs internalize the ligand (along with the pathogen or infected cell) for lysosomal processing, so that it can be presented on an MHC molecule to a naïve T lymphocyte.

3. Lysosomal proteases operate optimally below a pH of 5. Immature DC lysosomes have a pH of 5.5, while mature DC lysosomes have a pH of 4.5.

4. From the ER, a newly synthesized MHC II traffics through the Golgi to the lysosome. Targeting is directed by a lysosomal targeting sequence found in the invariant chain associated with MHC II.

5. Cystatin c.

6. MHC II is ubiquitinated at a conserved lysine residue in its cytoplasmic tail. This ultimately results in its localization to multi-vesicular bodies/lysosomes, where they reside, but are not degraded. This ubiquitination of MHC II is lost in mature DCs, hence its localization to the cell surface.

7. DCs express a variety of TLRs on their surface. Binding of one ligand to its TLR does not preclude continued binding of other ligands to TLRs between the same pathogen/DC pair. This combinatorial system of binding serves as a sort of “bar code” for the DC to read, and thus tailor an appropriate response. The cytokine(s) the DC subsequently secretes will guide the T cell response.

8. The newest T cell subset identified – regulatory T cells (Tregs).

3. Discussion Questions

1. MHC I is found in all nucleated cells and presents peptides to CD8 T cells, whereas MHC II is found only in antigen presenting cells (APCs) and presents peptides to CD4 T cells. Describe peptide loading of MHC I.
2. Describe peptide loading of MHC II.
3. How does cross presentation occur? Why is this useful?
4. Tolerance is a critical phenomenon that ensures that your immune system will not launch an immune response against your own cells. Describe how tolerance is achieved both prior to and after birth.

4. Answers to Discussion Questions

1. MHC I binds to antigens found in the cytoplasm. These antigens are from proteins synthesized by the cell's own machinery, e.g. viral proteins produced by the cell's protein synthesis machinery following viral infection. These proteins are degraded by the proteasome and the fragments are translocated into the ER by the TAP1/2 protein translocators. Peptides are then loaded onto MHC I and transported to the cell surface for recognition by CD8 T cells.

2. Following synthesis, MHC II traffics to the lysosome where it encounters antigens that have been endocytosed and degraded into peptide fragments. MHC II is usually associated with a chaperone, the invariant chain, which is partially cleaved in the lysosome, leaving a peptide fragment in the binding cleft of MHC II. MHC II interacts with a lysosomal molecule called HLA-DM, which destabilizes the invariant chain fragment, allowing the antigenic peptide to replace it in the binding cleft. Once loaded, the MHC II-peptide complex is transported to the cell surface, where it can bind to CD4 T cell receptors.

3. MHC I molecules usually present antigenic peptides derived from virally infected cells. In some cases, as with the Influenza virus, epithelial cells are most likely to be infected. If DCs are never infected, then these viral antigens will never be presented

to CD8 T cells by DCs in the secondary lymphoid organs. However, DCs have developed a process to circumvent this flaw: endocytosed material, which would conventionally be presented on MHC II, can escape the endocytic pathway and reach the cytosol. From there, this endocytosed antigen can follow the MHC I loading pathway.

4. Before you are even born, the bulk of self-reactive T cells have been induced to apoptose. The fetal thymus makes thymic epithelial cells and DCs that produce and present all the self antigens your body will ever produce. When TECs and DCs encounter T cells that recognize these self antigens, they signal for the apoptosis of those T cells. This process, though effective, is not 100% complete. After birth, the host utilizes Tregs to maintain tolerance. When DCs present peptide antigens in the absence of a pathogenic signal, they fail to release cytokines. Antigen presentation without the cytokine release activates Tregs, which inhibit the immune response.

5. Explain or Teach These Concepts to a Friend

1. Follow the life of an MHC-II protein from start to end.
2. Describe the cell biological processes that regulate lysosomal pH in DCs.
3. What are all the steps and interactions that are necessary to activate a T cell?

6. Research the Literature on Your Own

1. Sometimes the immune response goes wrong. Pick one of the auto-immune diseases mentioned in the lecture, or one of your own choosing, and understand the mechanism(s) underlying the disease.
2. Explore further the two processes that appear to regulate MHC-II transport; ubiquitination and association with the invariant chain.

7. Papers for Journal Club

Steinman, RM; Adams, JC; Cohn, ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. J Exp Med. 1975. 141:804-820.

This paper provides an historical perspective on dendritic cells and their discovery.

Jiang, A; Bloom, O; Ono, S; Cui, W; Unternaehrer, J; Jiang, S; Whitney JA; Connolly, J; Banchereau, J; Mellman, I. Disruption of E-Cadherin-Mediated Adhesion Induces a Functionally Distinct Pathway of Dendritic Cell Maturation. Immunity. 2007. 27:610-624.

Maturation of DCs is not achieved solely by encounter with a TLR agonist. Here, another mechanism of DC maturation is described.

Trombetta, ES; Ebersold, M; Garrett, W; Pypaert, M; Mellman, I. Activation of lysosomal function during dendritic cell maturation. Science. 2003. 299:1400-1403.

This paper describes in more detail the importance of lysosomal proteolysis, or the lack thereof, in MHC-II dynamics and DC function.

Chow, A; Toomre, D; Garrett, W; Mellman, I. Dendritic cell maturation triggers retrograde MHC class II transport from lysosomes to the plasma membrane. Nature. 2002. 418:988-994.

MHC-II transport is pivotal to DC function. This paper reveals, using microscopy, how MHC-II gets to the cell surface upon maturation, despite its localization in the proteolytic lysosomes.