IMMUNOLOGY

Cell mediated Immunity

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Cell Mediated Immunity

Generally refers to the immune response which involves cells of the host immune system. These cells are recruited based on their ability to recognize antigen and amplify cell reaction to the recognized antigen. However it must be understood that all immune responses are cellular. The separation into cellular immunity and Antibody / humoral immunity has been more for purposes of understanding the complex processes of immune responses. Antibodies are also derived from B cells and are a cellular product generated as a result of the involvement of antigen presenting cells and T cells. Cell mediated immunity is primarily of utility in defense against intracellular infections, whereas antibody response is effective against extra cellular microorganisms. Host immune response revolves around the T cell. T cell necessarily needs to interact with other cells such as dendritic cells, macrophages and B cells and infected host cells.

Pathogen recognition constitutes the genesis of all immune responses. The presence and expression of cell surface receptors constitute the basis of this recognition and specificity of interaction. Cells of the immune system express a wide variety of receptors, such as Toll like receptors (TLR), B cell receptor (BCR), T cell receptor (TCR), Major histocompatibility complex (MHC), etc. This wide array of receptors is used by the immune system to discriminate between self and non-self. The basis of recognition of non-self by each of these receptors differs. All receptors of the immune system are multi-chain complexes associated with signaling molecules and or accessory molecules that facilitate interaction between cells. For example the TCR is a dimer, consists of either $\alpha\beta$ / $\gamma\delta$ chains, associated with a CD3 signaling complex, and accessory molecules (CD4, CD8) and ligand for adhesion molecules etc. T cells recognize exclusively membrane bound antigen unlike antibodies which can react with free soluble antigen as well as cell associated antigen. The generation of membrane bound antigens is a function of antigen presenting cells such as dendritic cells, macrophages and B cells. Hence there is a fundamental difference in antigen perception by B and T cells.

There two major classes of cell associated MHC molecules, namely MHC I & II. MHC I is expressed on all nucleated cells, while the MHC II has a restricted distribution. It is present only on antigen presenting cells (APC's) such as dendritic cells, macrophages and B cells. Besides differences in distribution, the two molecules differ in their structural organization. MHC I consists of a heavy chain (α chain) associated with an invariant molecule known as β_2 microglobulin, whereas the MHC II molecule consists of two chains known as α and β chains.

The process of antigen being associated with MHC molecules by APC's is known as antigen presentation. Recognition of the antigen –MHC complex by T cells initiates a cascade of events leading to proliferation and differentiation into effector and memory T cells. The $\alpha\beta$ expressing effector cells of T cells are sub-divided into three functional populations, namely T Helper cells (T_H), Cytotoxic T cells (T_c) and T regulatory cells (T_{regs}). Each of these sub-populations can be distinguished based on specific cell surface markers. T_H can be differentiated from T_c / by the presence of the CD4 and CD8 membrane molecules, respectively. The expression of these molecules restricts the context in which these two populations of T cells recognize antigen, CD4+ T cells recognize antigen associated with MHCII, whereas CD8+ T cells recognize antigen associated with MHCI. T_{regs} are CD4+ and constitutively express an additional marker CD25. These cells regulate the activity of other T cells.

Antigen Presentation

T cell recognition of antigens requires antigen processing for reasons described earlier. Antigens are derived from invading extra-cellular or intra-cellular microorganisms. Elimination of these antigens is accordingly dealt by 2 different pathways. Exogenous antigen i.e. antigen taken up endocytosis is processed by the endocytic pathway and displayed on the cell surface in association with MHC class II. Antigens of endogenous origin are processed by the cytosolic pathway and the processed antigen is presented along with MHC I. Experimental evidence clearly showed the presence of these two pathways. Class I presentation requires the endogenous synthesis of pathogen derived (virus) proteins, whereas, Class II presentation is independent of protein synthesis, in fact depends on peptides generated as a result of intracellular degradation of exogenous proteins. Hence peptides presented by Class I and II are generated by distinct intracellular compartments and pathways. The pathway for processing of endogenous antigens is known as cytosolic pathway; whereas the pathway for processing of exogenous antigens is known as the endocytic pathway.

The Cytosolic Pathway

This pathway is intimately associated with the regulation and turn over of intracellular proteins. Normal and abnormal proteins are degraded into their constituent amino acids by exopeptidases, some peptides may survive this degradation and are available for association with MHC I. The mechanism of selection of these peptides is unclear. The proteolytic system in the cell is multi unit, barrel shaped structure known as a proteosome. Peptides generated in the cytosolic compartment are transported to the rough endoplasmic reticulum to generate MHC I-peptide complexes. The transport is mediated by proteins known as TAP1 & 2 (Transporter associated with antigen processing). These heterodimeric proteins are bound to the membrane of the rough endoplasmic reticulum. The translocation of peptides generated in the cytosolic compartment into the RER by TAP requires ATP hydrolysis. The extra long peptides are trimmed to optimize its interactive ability with MHC I, as the optimal peptide length is 9 amino acids. The bringing together of the freshly synthesized MHC I molecules and peptides is facilitated by chaperone molecules.

The Endocytic pathway

Endocytosed antigen is degraded to generate peptides. The compartments wherein the degradation occurs are acidic. They include early and late endosomes, and lysosomes. The package of enzymes contained within these vesicles include a range of acid dependent hydrolases, phosphatases, lipases, proteases, nucleases etc. As antigen presenting cells express both MHC I and MHC II, there exists a mechanism that prevents the same peptide that associates with MHC I to bind with MHC II. On completion of the synthesis of the class II MHC molecule it is associated with a protein called invariant chain. This protein prevents the binding of endogenously derived peptides with MHC II. The invariant chain is important for folding of the α and β chains, i.e. helps in MHC molecule maturation, and in transportation of the MHC molecule through RER to the Endocytic compartment. The presence of high proteolytic activity degrades the invariant chain to a short peptide known as CLIP (Class II associated invariant peptide). CLIP is located in the peptide binding groove of MHC II. The groove of MHC II can accommodate 13- 18 amino acid length. Non-classical MHC molecules such as HLA-DM expressed in Endocytic compartments of antigen presenting cells facilitates peptide loading on to

the MHC molecule. The high acidic conditions in the lysosome permit the exchange of the CLIP with antigenic peptide. With the formation of the MHCII- peptide complex, it is transported to the surface where at neutral pH it assumes a stable configuration.

Some cells such as dendritic cells are capable of cross presentation of exogenous antigens, i.e. peptides derived from antigens endocytosed some times be processed by both MHC I and II pathways. This has an advantage as the antigen presenting cells to engulf viruses, process viral proteins, leading to the generation of cytotoxic T cells.

Presentation of Non-peptide antigens: T cells are also capable of responding to non-protein antigens, this is characteristic of the γδ T cells. These cells proliferate in response to glycolipid antigens of M. tuberculosis. The antigen presentation of non-protein antigens is mediated by a family of molecules known as CD1 molecules. They are similar in structure to MHC I molecules. Recent evidence suggests that the pathway of antigen processing for CD1 antigen presentation appears to be distinct from the more well understood Class MHC I & II pathways. The interrelationship between the pathways is an area of active investigation.

T – B cell interaction

B cells unlike T cells differ in their perception of antigen. The recognition is mediated by the BCR complex, which consists of the membrane bound immunoglobulin (mIg) and associated signaling complex. B cells can directly recognize antigen unlike T cells. Mere recognition of antigen by mIg does not lead to differentiation, proliferation, generation of effector / memory cells; this requires interaction with T_H cells and the presence of cytokines. The class of antigens involved in the activation of B cells with T cell help is known as thymus-dependent antigens. Unlike macrophages and dendritic cells B cells are capable of presenting antigen when present in limiting quantities. The reason for this is that an antigen internalized by B cells is determined by the specificity of receptor present on the B cell.

There are several steps described following thymus-dependent antigens binding to B cells leading to B cell activation. Following binding of antigen, it is internalized by receptor mediated endocytosis and undergoes the process of antigen processing and presentation as described earlier. Concomitantly following antigen association with the BCR, leads to up regulation of MHC and co-stimulatory molecules (B7). The presence of these two molecules augments the ability of the B cell to function as an antigen presenting cell. The recognition of the MHC-Ag complex by T cells leads to the formation of T-B cell conjugates. It must be understood that only antigen specific T cells form conjugates. Conjugate formation leads to intracellular structural alterations in both cells, which would aid in focusing the release of T cell derived cytokines on the B cell and the receptivity of the B cell to respond to these cytokines, present in the conjugate. This concentrated release of cytokines facilitates the activation of the antigen specific B cell.

Besides release of cytokines, membrane contact between the two interacting cells is the expression cell surface molecules such as CD40L (CD154) on T cells and CD40 on B cells, facilitate the generation of an essential signal for T cell dependent B cell activation. This signal in (2^{nd} signal) in tandem with the signal generated with the signal (1^{st} signal) generated by the cross linking of the mIg by the antigen are essential for B cell activation. This is followed by a

series of intra-cellular signaling events, leading to generation of transcription factor(s) such as NF-κB, followed by activation and expression of several genes. Following activation B cells express cytokine receptors such as IL-2R, IL-4R. IL-5R etc.. Cytokines made available by T cells bind these receptors, leading to the generation of a third set of signals. These signals initiate B cell proliferation, differentiation into plasma cells, memory B cells, Ig class switching and affinity maturation of Igs.

Role of Cytokines

Cytokines are small molecular weight regulatory glycoproteins generated by cells in response to stimuli / stress. Cytokines are generated by a host of cells but the cardinal sources are T_H cells, dendritic cells and macrophages. Cytokines produced by these cells influence a whole network of immune and non-immune cells. The discussion here will be limited to the role of key cytokines associated with B cell physiology, namely IL-2, IL-4, Il-5 and IFN-γ.

Cytokine	**Source**	**Biological effect**
IL-2, 15-20 kDa, T cell growth factor	**T cells**	T , NK and **B cell proliferation and differentiation**
IL-4 , 15-19 kDa, B-cell stimulating factor (BSF-1)	**T_H2 cells**, mast cells, bone marrow	T and **B cells growth and development** , **Facilitates Ig isotype switch to IgE**
IL-5, 45 kDa, eosinophil differentiation factor / colony stimulating factor	**T_H2 cells**, mast cells, eosinophils	Induces eosinophil formation and differentiation, **B cell proliferation**
IFN-γ, 17-40 kDa, macrophage activating factor	**T_H1 cells**, NK cells, CD8 T cells	Macrophages, T, NK and **B cells Activation, growth and differentiation** and, **Blocks IgE and facilitates IgG production**

As can be seen from the above table cytokines have numerous functions. These functions can be classified as pleiotropic, redundant, synergistic and antagonistic. These functions are exemplified in the role of cytokines that influence B cell activation, differentiation and proliferation. For example the biological effect of IL-4 is pleiotropic, as it induces and facilitates proliferation of B cells as well as thymocytes and mast cells. This effect is in consort with other cytokines namely IL-2 and IL-5 which also induce proliferation of B cells, an example of redundancy in cytokine function. IL-4 and IL-5 together facilitate Ig class switch to IgE, hence they act synergistically.

The blocking of Ig class switch to IgE by IFN-γ, is a classical example of the antagonistic role of cytokines.

Immune Tolerance

The unresponsiveness to a given antigen is known as tolerance. Hence distinct from generalized immune suppression as tolerance is antigen specific. Numerous mechanisms have been attributed to the establishment of tolerance. The antigens that induce tolerance are known as tolerogens. This feature is not necessarily limited to the nature of the substance but also determined by distortions / aberrations in the physiological events associated with the failure to generate an immune response. The factors that associated with the antigen, such as: antigen dosage; half life of the antigen; route of antigen administration; and host related factors, such as genetic predisposition, inappropriate / inadequate T cell stimulation etc. Besides antigen sequestration has been considered as a means of inducing tolerance. Physical separation of the antigen from the immune system such as the blood brain - barrier, antigens expressed during adolescence are all examples of absence of immune response to self antigens, as long as the barriers are present. Any breach of these barriers would lead to initiation of immune response.

Besides the above mentioned features of tolerance and its induction, as a part of the normal gamut of immuno-competent cells in healthy individuals self reactive lymphocytes are present. Presence of these cells does not always result in their activation leading to auto-immune reactions, hence their activity is must be regulated. This regulation is mediated by cell death, cell anergy, regulation of T cell activity by T_{reg} cells.

The essential mechanism in establishing tolerance is deletion of self reactive lymphocytes during the ontogeny of immuo-competent cells in a host. Hence this is a regulatory mechanism which operates during the development and expression of functional receptors namely TCR and BCR. This critical process is known as central tolerance process that eliminates self-reactive T & B cells in the bone marrow and thymus respectively. Central mechanisms known to regulate & establish tolerance, it is mot complete, as self reactive cells have been demonstrated in peripheral compartments of the immune system. Inhibition of these cells in the periphery is modulated by a process known as peripheral tolerance.

Significant progress in the understanding of the mechanisms of tolerance has come about by the use of transgenic mice. Transgenic mice are mice in whom a foreign gene has been incorporated into its genome and is functional. Two sets of transgenic mice were taken, one that expressed specific for hen egg lysozyme (HEL) Ig were crossed with transgenic mice expressing HEL. It was found that the F1 generation mice lacked B cells expressing anti-HEL antibody. It was later demonstrated that the deletion of B cells expressing anti-HEL antibody were eliminated in the bone marrow. Besides elimination B cells also undergo a process of receptor editing, wherein the antigen specific regions of the receptor are replaced by alternate gene products. The elimination of auto-reactive cells is not only by receptor editing, but also by clonal deletion and apoptosis.

As the process of elimination of self-reactive cells in the central compartment is not fool proof, some self-reactive cells appear in the periphery. This could be due to a variety of reasons such

as: absence of the specific self antigen in the thymus / bone marrow; interaction with self antigen is weak, hence does not induce deletion, and these weakly interactive cells escape into circulation. The mechanism of inactivation of these cells in the peripheral compartment is known as peripheral tolerance.

Examining transgenics expressing HEL, it was observed that T cells that recognize HEL were eliminated in the thymus. B cells reactive with HEL are present in the periphery. these B cells on encountering HEL become unresponsive or anergic, owing to the lack of T cell help. Besides absence of T cell help, the role of co-stimulatory molecules have been demonstrated, which include T cell activating co-stimulatory complexes CD28-B7 and inhibitory complexes namely CTLA-4 –B7. The importance of CTLA-4 in tolerance was recognized in knock out mice, wherein in these mice un-regulated proliferation of lymphocytes was observed, leading to development of auto-immune disease. Besides these two mechanisms of inducing peripheral tolerance, it can also be induced by Tregs. These class of cells down regulate inflammatory responses in secondary lymphoid organs. These are generated in the thymus during T cell development. Potential T cells in the thymic environment depending on their affinity for self antigens meet their destiny. Highly reactive T cells are eliminated, cells with low affinity undergo further maturation or the cells with intermediate affinity express Foxp3 (transcription factor) ad differentiate into $T_{regs.}$

Auto-immune disease

Failure of tolerance processes to regulate self reactive lymphocytes leads to auto-immune disease. Auto-immune diseases may be organ specific, or systemic. The auto-immune diseases may be mediated by auto-antibodies, or by the release of sequestered antigens, or molecular mimicry.

In an auto-immune disease such as Hashimoto's thyroiditis the patient generates auto reactive T_H cells and auto- antibodies to thyroid components. The condition leads to lymphocyte infiltration and inflammation, resulting in enlargement of the thyroid gland,(Goiter). Hypothyroidism is caused in these individuals by the generation and presence of auto-antibodies that react with proteins (thyroglobulin) that are required for iodine uptake.

One of the best examples of systemic auto-immune disease is systemic lupus erythematosus (SLE). In these individuals auto-antibodies are generated to a wide array of antigens. These auto-antibodies form complexes which deposit along blood vessels, lead to hypersensitivity reaction. These complexes will activate complement resulting in damaged vessel walls, leading to vasculitis and glomerulonephritis. Besides excessive complement activation and generation of its breakdown products leads to neutrophil aggregation on the vasculature , leading to occlusion of blood vessels, resulting in wide spread tissue damage.