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CURRENT ADVANCES IN MOLECULAR IMMUNOLOGY: REFERENCE GUIDE FOR REVIEWS ON MOLECULAR VACCINES

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1. ABSTRACT

The field of immunology has made significant and rapid advances in the past two decades. This understanding has led to a systematic approach to studying the various aspects of development, activation, expansion, maintenance and termination of immune responses, with respect to disease-pathogenesis, prevention and therapeutic interventions. This issue of the journal focuses on reviewing the molecular aspects of vaccine design. In order to provide the reader with a framework and reference to the new aspects of immunological concepts, this article reviews the recent developments in immunology, with emphasis on induction of immune responses during vaccination. Key recent reviews in each section are provided for the readers to obtain detailed information. Several schematic figures are provided for a visual representation of basic concepts.

2. THE INNATE IMMUNE RESPONSE

The importance of innate immunity has not only been recognized as the first barrier to disease, but also in cooperation and regulation of the ensuing adaptive immune response. In the induction of adaptive immunity, processing and presentation of antigens is followed by recognition of antigen specific receptors, which leads to activation of and differentiation to effector cell-mediated and humoral immune responses. (1-3)

Innate immunity provides the first line of defense by the host. These responses were historically deemed non-specific, mediated by inflammatory responses, such as cytokines and prostaglandins. Innate immunity is mediated by cells such as phagocytes and natural killer cells, circulating proteins, such as components of the complement system, and numerous anti-microbial peptides. Recent studies have demonstrated that the innate responses also recognize specific elements of pathogens. These components utilize germline-encoded "pattern recognition receptors" (PRR) to recognize molecular structures, or "patterns," present on various classes of microbes. Many of these receptors, such as the Toll Receptor family of proteins, are conserved throughout evolution and serve the same function of anti-microbial defense in all multicellular organisms, including plants.

2.1. "Specific" interactions in innate immunity

Lipopolysaccharides (LPS) on bacterial membranes have been studied extensively due to their ability to induce a burst of cytokines resulting in septic shock. A serum factor, LPS binding protein (LBP), co-ordinates the interaction of LPS with the glycosyl-phosphatidyl inositol-anchored, CD14. Binding of the microbial

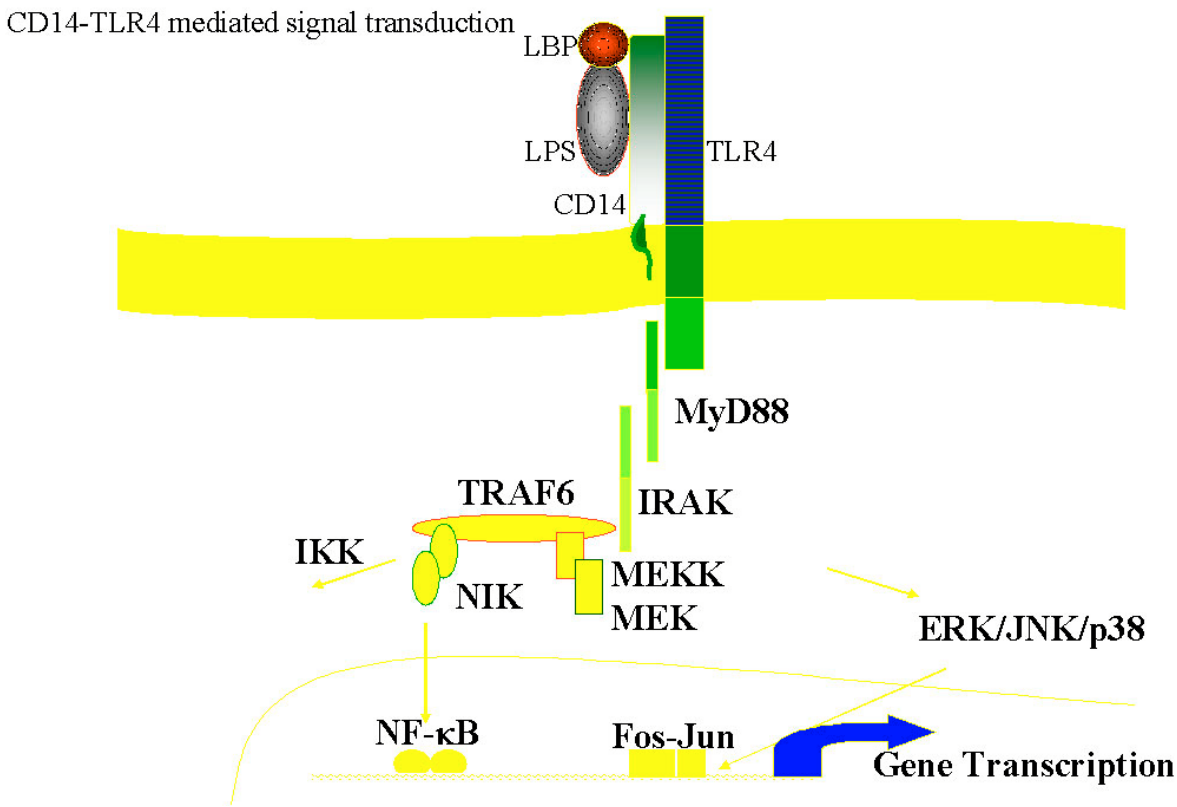

Figure 1. Signaling events following innate immune response. Binding of lipopolysaccharide (LPS) and LPS-binding protein (LBP) initiates a signaling cascade, by recruitment of Toll-like receptor 4 (TLR4). Down stream signaling through TLR has been shown to involve adaptor proteins MyD88, IL-2R accessory protein kinase (IRAK), TNFR associated factor 6 (TRAF-6) and NF-kB inducing kinase (NIK) leading to activation of NF-kB and subsequent gene transcription. In addition, MAP-ERK kinase kinase (MEKK) and MEK pathways activate Mitogen Activated Protein kinases - ERK, Jun-N-terminal kinase (JNK) and -38-kinase, leading to activation of transcription factors Fos and Jun. These signals lead to expression of various genes such as inflammatory cytokines, costimulatory molecules, chemokines, etc.

products to CD14 triggers production of various pro-inflammatory cytokines including IL-1 and TNFalpha. IL-1 and TNFalpha bind to their cognate receptor and initiate a signal transduction cascade culminating in activation of transcription factors, such as NF-kB, which activates a range of cytokine and receptor genes. Activation of innate responses through the TLR leads to recruitment of inflammatory cells, such as macrophages and neutrophils, and enhancing phagocytosis and killing of microorganisms. A family of Toll-like receptors (TLR), first identified in Drosophila, cooperates with CD14 to transduce signals (4). TLRs have been recently suggested to play a major role in the innate immune responses against microbial pathogens in mammals. Mammalian TLR family members are transmembrane proteins containing repeated leucine-rich motifs in their extracellular regions. Mammalian TLRs also contain a cytoplasmic region homologous to IL-1 receptor (IL-1R) and can transduce intra-cellular signals (Figure 1).

Expression of TLR on macrophages, B cells, T cells and dendritic cells is consistent with their role in regulating immune responses. Signaling through TLR has been shown to induce inflammatory cytokines such as IL-1, IL-6, IL-8, IL-10, IL-12 and TNFalpha and costimulatory molecules on antigen presenting cells. Expression of these molecules on adipocytes, dermal and mucosal epithelial cells, supports the hypothesis that these molecules may be involved in the "first line of defense" against invading pathogens. In addition of inducing activation of genes which regulate expression of anti-microbial peptides, TLR signals also induce activation of inducible Nitric oxide synthetase (iNOS). More recently, TLR9 has been shown to mediate signals to bacterial methylated CpG DNA (5) CpG DNA sequences have been implicated in enhancing immune responses mediated by DNA vaccines. In summary, interactions of components of pathogens with evolutionarily conserved molecules in the immune system, underscores the importance of innate immunity in protection against pathogens. Studies of innate immune responses and its influence on adaptive immunity have been in extensive investigation for designing novel mechanisms of adjuvants for vaccines (6). In this respect, the review on Adjuvants for vaccines in this issue of the journal gives an overview of innate immune responses in vaccine design.

3. THE ADAPTIVE IMMUNE RESPONSE

The adaptive immune response has evolved to specifically recognize "foreign" antigens. The recombination

process of immunoglobulin and T cell receptor genes, which establishes diversity of T and B cells, has recently been reviewed in a special issue of Cell (7). The sequence of induction of immune responses involves processing and presentation of antigens, activation and regulation of T and B cells, and induction of longer term memory responses. The following description of events involved in induction of adaptive immune responses focuses on recent findings on these processes.

3.1. Processing and presentation of antigen

Capture, processing, presentation and trafficking of the antigen play central roles in induction, and persistence of immune activation and memory. Extensive studies on the mechanism of processing of antigen by major histocompatibility complex (MHC) class I and MHC class II have elegantly established the biochemical pathways involved in displaying peptides to T cells (8-11). Most intracellular proteins are degraded by "immuno"proteosomes into oligopeptides, which are transported by MHC class I molecules to the surface, to be presented to CD8+ cytotoxic T lymphocytes (CTL) for recognition by the T cell receptor. Immunoproteosomes have been shown to affect the generation of antigenic peptides and their composition varies in cell types, leading to cell-type specific generation of CTL. MHC class I molecules bind peptides in the endoplasmic reticulum. Recent discovery of ER aminopeptidases (ERAP), has further delineated by precise mechanism by which proteins are processed into 8-11 aminoacid length peptides, which is the absolute requirement for fitting in the groove of the MHC class I molecules (12, 13). A ground-breaking discovery has also very recently been described in MHC class I processing which shows a mechanism of protein splicing, which has implications in exponential diversity of antigenic epitopes presented by APC(14). These recent discoveries of the mechanism of MHC class I processing pathways, will be crucial both in vaccine design and development of assay systems to accurately measure CD8 CTL functions.

In contrast extracellular antigens are directed towards MHC class II antigen-processing and presentation pathways, which involve peptide loading in the endosomal compartments. Expression of MHC class II molecules is tightly regulated by MHC class II transactivator (CIITA) on professional APC, primarily DC, macrophage-lineage cells and B cells. Recent advances in MHC class II gene regulation involve studies in chromatin remodeling, histone acetylation-deacetylation of the SW1/SNF complexes, and CpG methylation, which are critical in restricting expression of these proteins to professional APC. Progress in the understanding of MHC class II processing include regulation of the peptide loading by chaperones HLA-DO and HLA-DM, and the modifications of the MHC class II compartment (MIIC) during activation processes of cells (10). In this respect, immature DC, which are efficient in endocytosing extracellular antigens, are not efficient in antigen processing. Maturation of DC results in redistribution of MHC class II compartments, resulting in increased degradation of proteins, and increase efficiency of antigen processing pathways (15, 16).

Significant advances have been made in the understanding of processing and presentation of lipid fatty acids, glycolipid and lipopeptide antigens, such as lipid antigens of Mycobacteria and galactosylceramide, by CD1 molecules. CD1 molecules have a unique hydrophobic binding groove that binds lipid antigens in both secretory and endosomal compartments of the APC (17, 18). CD1 isoforms sample antigenic epitopes from early and late endosomes and lysosomes by differential tracking which is mediated by tyrosine-based motifs in the cytoplasmic tails. Loading of antigens in the CD1 groove, involving acyl chains of lipids, has been demonstrated to involve lipid exchange between CD1, lipid and endosomal lipid transfer proteins, on the basis of their differential affinities for lipids. The importance of CD1 molecules in processing lipid antigens have been genetically established in knockout mouse models, and more recently in the Hermansky-Pudlak syndrome type 2. In this human disease, there is a defect in CD1-lipid antigen presentation, while MHC class II functions are retained (16).

Although MHC restriction has been firmly established as the mechanism of presentation of non-self antigen by self MHC molecules, the alternate mechanism of presentation of MHC mismatched cells has also been demonstrated to present antigens. The mechanism involved in the later process, termed "cross-priming" shows that MHC class I molecules expressed on the cell surface are capable of presenting peptide derived from exogenous protein antigens. This process has been shown to require proteosomal degradation of exogenous proteins to (Transporter of Antigenic Peptides) TAP-dependent endoplasmic reticulum retro-translocation, where peptide loading occurs via the MHC class I pathway. The complex biochemical studies of trafficking of peptides in cellular organelles have suggested the mechanism of cross-priming involves direct transfer of proteins from micro pinosomes to the cytosol. Furthermore, the phagosomes themselves have been recognized as antigen presenting organelles for cross-priming due to recruitment of ER membranes during phagosome organelogenesis (19). The Sec61 complexes play a significant role in recruitment of peptides into this MHC classes I containing phagosome (20, 21). In this respect, Sec61 plays in role in retro-translocation of β2-microglobulin from the ER to the cytosol, through an ATP-dependent mechanism. Cross-priming, previously considered a rare observation, has been demonstrated to be a robust phenomenon, involved in several antigenic systems (22).

Figures 2-5 schematically depicts the MHC class I MHC class II, CD1, and cross-priming pathways of antigen processing and presentation. Readers are encouraged to read reviews on antigen presentation for detailed descriptions. The threshold of MHC-peptide complexes on the surface of APC determines the formation of the immunological synapse, and the strength of signals delivered to T cells, which ultimately determines the nature of the immune response. Understanding the mechanisms of antigen processing and

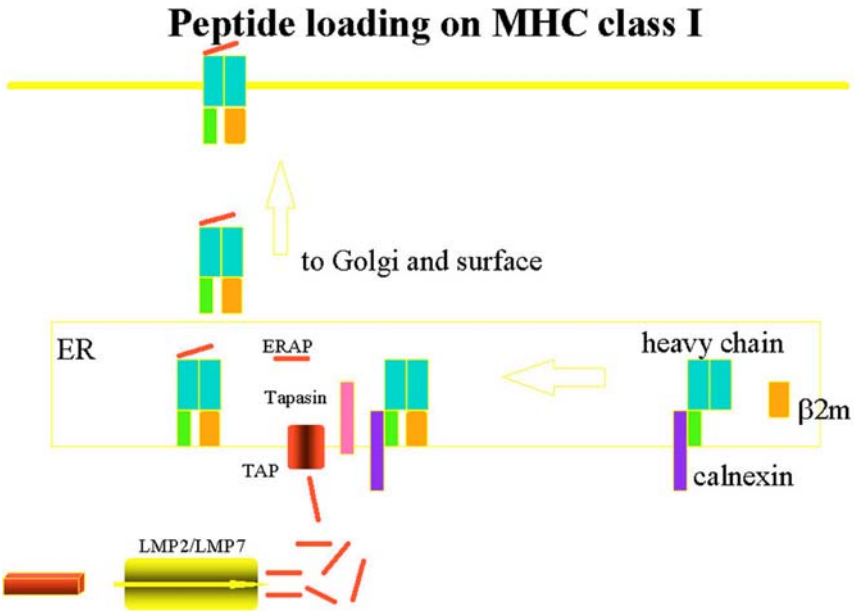

Figure 2. Peptide loading on MHC class I. MHC class I heavy chain protein is synthesized in the cytoplasm and transported to the Endoplasmic Reticulum (ER). Calnexin (chaperone) assists in folding of MHC class I. Binding of β2 microglobulin (β2m) to MHC class I releases calnexin. Cytosolic proteins are degraded by the proteosome (LMP2/LMP7) into peptides, which are led into the ER through the Transporter for Antigenic Peptides (TAP). Tapasin assists in peptide transfer/loading from TAP to MHC class I. A newly discovered protein (ERAP [endoplasmic reticulum aminopeptidase]) plays a major role in trimming peptides to 8-11 aminoacids, to fit into the MHC antigen binding pocket. Upon appropriate loading of peptides into the MHC class I antigen binding groove, the complex is transported through the Golgi to the cell surface.

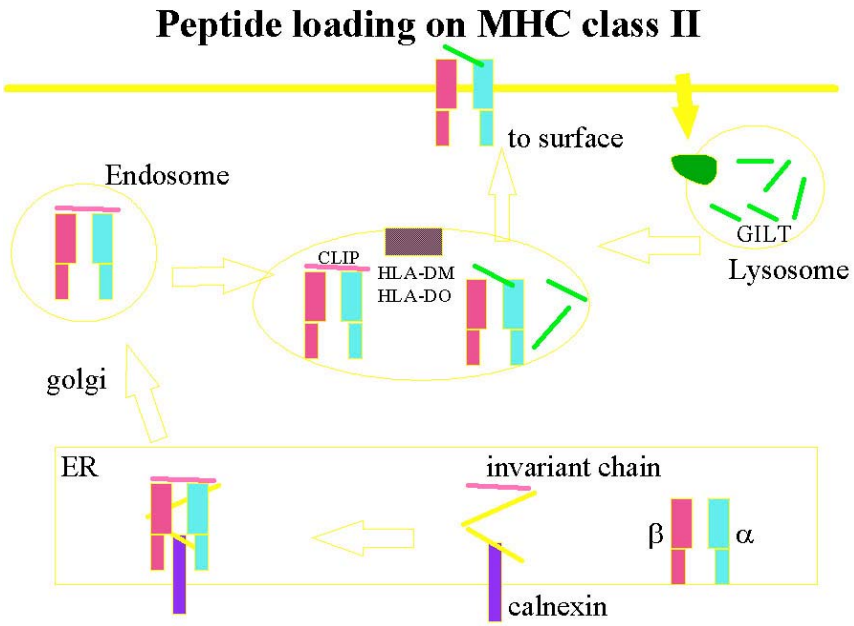

Figure 3. Peptide loading on MHC class II. Exogenous proteins are taken up in lysosomal vesicles and gradually degraded into peptides by several proteolytic enzymes. Recently identified enzyme, GILT [gamma interferon inducible lysosomal thiol reductase), plays a significant role in generation of appropriate peptides for presentation. MHC class II molecules are synthesized in the cytoplasm, translocated to the ER. The nascent αβ heterodimers associate with the invariant chain. The invariant chain plays a role as a chaperone, as well as blocks the binding of endogenous peptides in the ER by itself binding to the peptide groove. The invariant chain is degraded in the endosomal compartment; leaving a class II associated invariant peptide (CLIP) in the antigen-binding groove of MHC class II. MHC class II containing endosomes fuse with the peptide containing lysosomal vesicle. Peptide loading on MHC class II is facilitated by HLA-DM and HLA-DO. Appropriately leaded MHC class II molecules are transported to the cell surface.

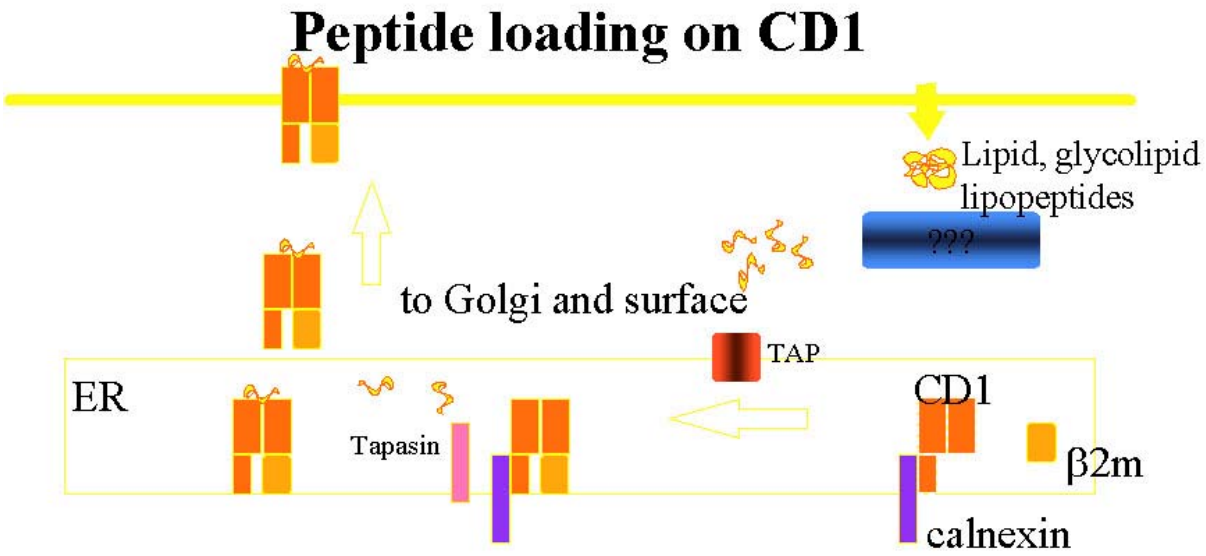

Figure 4. Peptide loading on MHC class II. Lipid, glycolipid and lipopeptide antigens are taken by by cells through various mechanisms including pinocytosis, membrane fusion etc. These moieties are broken down into smaller components in the cytoplasm, by yet to be defined processes. The peptides enter the ER through a TAP-dependent pathway, and are loaded on to CD1 molecules. CD1 molecules have a unique hydrophobic binding groove that binds lipid antigens in both secretory and endosomal compartments of the APC. CD1 isoforms sample antigenic epitopes from early and late endosomes and lysosomes by differential tracking which is mediated by tyrosine-based motifs in the cytoplasmic tails. Loading of antigens in the CD1 groove, involving acyl chains of lipids, has been demonstrated to involve lipid exchange between CD1, lipid and endosomal lipid transfer proteins, on the basis of their differential affinities for lipids.

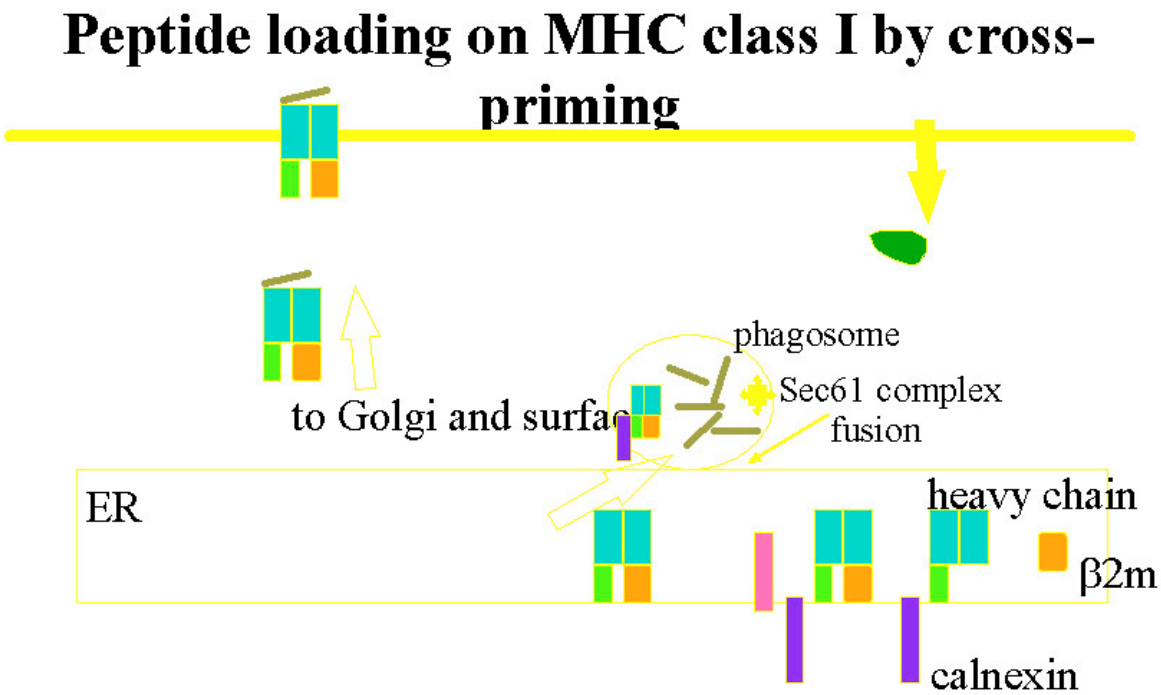

Figure 5. Peptide loading on MHC class I by cross-priming. The mechanism involved in cross-priming shows that MHC class I molecules expressed on the cell surface are capable of presenting peptide derived from exogenous protein antigens. This process has been shown to require proteosomal degradation of exogenous proteins to (Transporter of Antigenic Peptides) TAP-dependent endoplasmic reticulum retro-translocation, where peptide loading occurs via the MHC class I pathway. Phagosomes themselves have been recognized as antigen presenting organelles for cross-priming due to recruitment of ER membranes during phagosome organelogenesis. Sec61 plays in role in retro-translocation of β2-microglobulin from the ER to the cytosol, through an ATP-dependent mechanism.

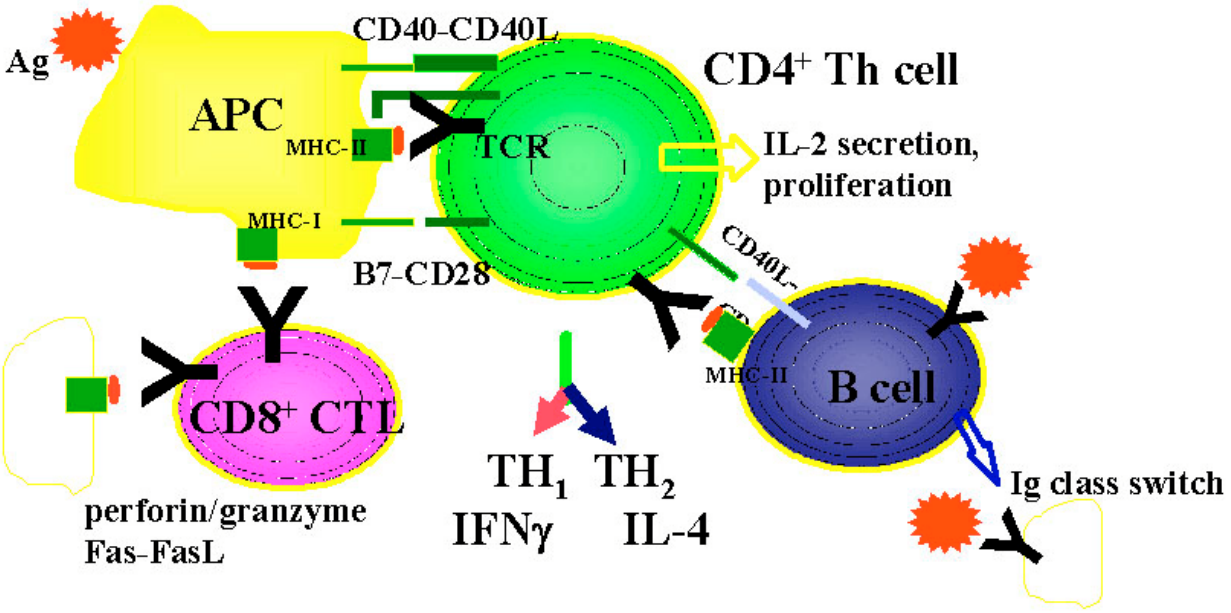

Figure 6. Induction of immune responses. Antigen taken up by antigen presenting cells (APC) is processed and presented by MHC class I to CD8 T cells, and MHC class II to CD4 T cells. Recognition of the antigen, along with costimulatory molecules (B7-CD28; CD40-CD40 ligand) results in activation of antigen-specific CD4 T cells, which leads to lymphoproliferation and cytokine secretion. Depending on several conditions (e.g. strength of antigen signaling, costimulation, cytokines secreted by APC, etc.) CD4 T cells differentiate into either Th1 or Th2 type cells. Th1 cells secrete predominantly IFNgamma which plays a role in activation of cell mediated immune responses which culminates in activation of cytotoxic T lymphocytes (CTL). CTL mediate effector cell functions by mechanisms involving Fas-FasL and perforin-granzymes. Th2 cells, on the other hand, secrete IL-4, which helps B cells differentiate into antibody secreting plasma cells.

presentation by APC will give insight into the development of antigen delivery systems (vectors) for induction of effective immune responses.

3.2. Activation of T cells

3.2.1. The Initiation of immune responses: cell-cell interactions

Following recognition of antigen by T lymphocytes through interactions of the TCR with antigen-MHC complex leads to a sequential cascade of positive and negative signals that culminate into effector functions (23-26). In order to ensure that immune responses develop in a regulated manner, naïve lymphocytes need at least two signals to be fully activated to proliferate and differentiate into effector cells. Signal 1 is provided by specific antigen the TCR binding to the antigen-MHC complex. Rendered alone, this signal is insufficient to stimulate a response. Conversely, when induced by itself, signal 1 can induce a state of unresponsiveness or anergy. However, when the antigen is delivered along with the second signal, complete activation of effector T cell functions ensue. The best defined second signals for T cells are CD28, through the interaction with its ligands, B7-1 (CD80) and B7-2 (CD86). Expressed on professional APCs, such as dendritic cells and macrophages, their expression peaks after the activation of APCs. Activated APCs also produce cytokines during innate immune reactions, which further stimulate T cell responses. The B7 proteins are recognized by the CD28 receptor, which is expressed on most naïve T cells, especially those of the CD4 subset. Together with the antigen receptor signal (signal 1), interaction with B7 proteins by CD28 (signal 2) leads to T cell activation, clonal expansion, and the development of effector T cell function (Figure 6). Activated T cells express CD40 ligand (CD40L), which in turn activates APCs to increase expression of B7 costimulatory molecules. In addition CD40-CD40L interactions are critical for production of cytokines, such as IL-12 by APC, that induce the differentiation of T cells. CD40L serves to amplify T cell proliferation and differentiation into effector cells. In the past 5 years, with the advent of sequencing of the human genome, and access to novel sequence databases, there has been an explosion of molecules identified on the surface of APC and T cells which are being studied for the criticality in the sequential cascade of cell surface molecules. Figure 7 shows some of the molecules and their ligands which are under investigation in the T cell regulatory process.

With the advents of new imaging technologies, the role of cell surface molecules in intercellular communication is being unraveled. The term "immunological synapse" has been descried as the junction between two cell types. It involves the formation of a flattened stable interface, allowing for highly organized dynamic structures that can provide a platform for a bidirectional flow of information between cell types (26).

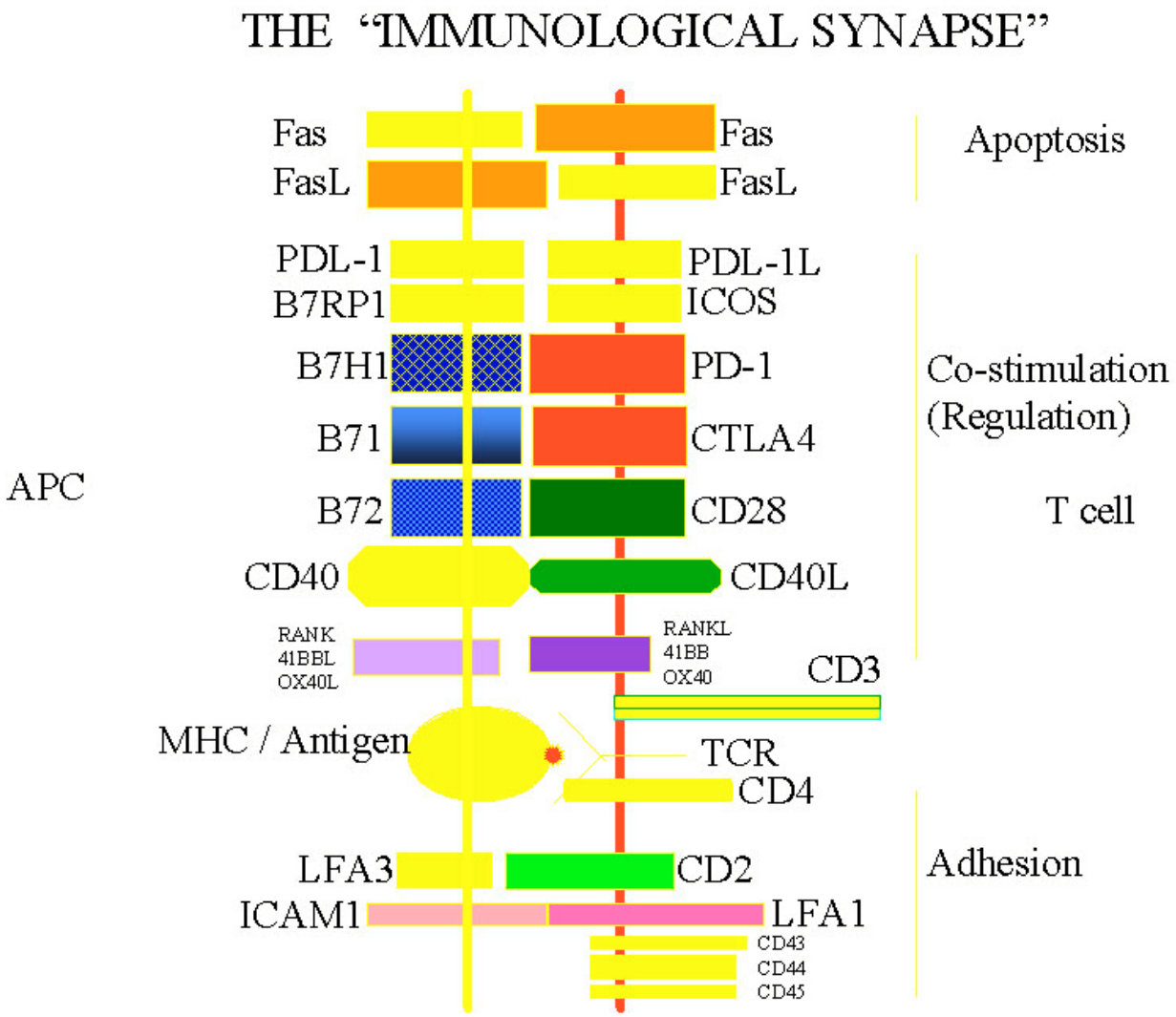

Figure 7. The "immunological synapse". T cell antigen receptors (TCR) interact with MHC-peptide complexes in the immunological synapse between T cells and antigen presenting cells (APC). CD3, CD4/CD8 molecules play roles in both adhesion and signaling in T cells. Adhesion molecules, such as Leukocyte Function Antigen (LFA3) with bind to CD2, and Intracellular Adhesion Molecules (ICAM1) which bind LFA1, enhance the binding efficiency of cells. Costimulatory molecules listed play roles in transducing positive and negative signals in a bidirectional manner. Molecules for termination of immune responses transduce apoptotic signals.

3.2.2. The nature of the biochemical signals that activate T cells

Cell-cell interactions induced by interactions of several molecular ligand-receptor pairs result in aggregation of molecules, and formation of lipid rafts which lead to development of an immunological synapse at the antigen recognition site (27-29). This aggregation of receptors brings signaling molecules into close proximity, and achieves the threshold for activation of intracellular kinases and phosphatases. In this respect, elegant studies have delineated the complex biochemical pathway of activation of enzymes and substrates which culminate into activation of transcription factors which translocate to the nucleus. In general, signal transduction in various cell types is fundamentally similar, such as activation of kinases and phosphatases, increase in intracellular calcium, and activation of mitogen activated kinases. Adaptor molecules scaffold the pathway, and are critical in emaculate regulation of the signaling pathways. These cytoplasmic biochemical messengers of signals eventually result in activation of transcription factors which bind to and regulate gene expression of a wide variety of proteins including cell surface molecules, and soluble cytokines and chemokines. These in turn play essential roles in cell-cell communication processes and mediate effector cell functions. Figure 8 schematically shows the signaling molecular cascade mediated through the TCR and CD28 molecules. Studies with gene expression profiles will allow for further delineation of the signaling cascade, through the T cell receptor and costimulatory molecules, which will be crucial in understanding the pathways for regulation of T cell functions.

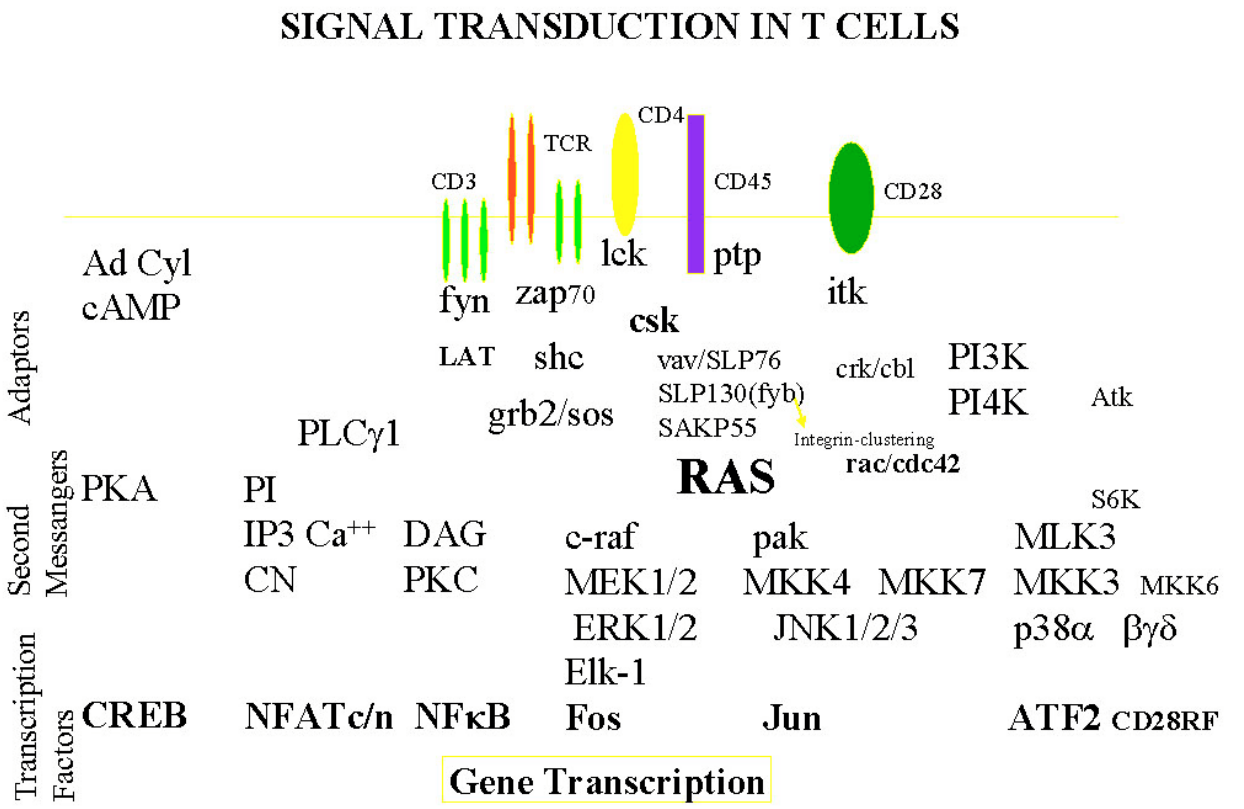

Figure 8. Signal transduction in T cells. The TCR activates transcription-factor families which are responsible for regulation of gene expression. In addition, activation of signaling pathways are important for amplification of the signals initiated after formation of an immunological synapse. The importance of this concept is that these signaling pathways play an important role in creating the physical environment for sustained signaling. For detailed descriptions of pathways, please refer to the references noted in the section in the text.

3.2.3. Regulation of T cell responses

After recognition of antigen, T cells undergo proliferation and differentiation, which culminates in cytokine secretion. The regulation of cytokine secretion by T cells has been central to induction of cell-mediated and humoral-immune responses. (30-32). The understanding of the mechanism of the processes (biochemical, molecular and cellular) involved in decisions for commitment to secretion of "linked" cytokines has been studied extensively. The pattern of cytokines secretion by T cells has been utilized to define subsets of T helper (Th) cells into Th1-type (IFNgamma secreting) and Th2-type (IL-4 secreting). The complexity of the network of cytokines, and their interrelated functional effects on T cells is exemplified by IL-2.

3.2.3.1. Study of IL-2: case in point for unraveling the complexity of cytokines in regulation of immune responses

IL-2 was the first soluble factor to been identified as a key cytokine to promote T cell growth. In the recent years however, cytokines which utilized the IL-2 receptor γC chain receptor, i.e. IL-4, IL-9, IL-15, IL-21, have all been shown to be vital for cell growth and differentiation at different stages of T cell differentiation. The initial studies demonstrated that IL-2 played a key role in cell growth has recently been challenged. First, cyclosporin A, which inhibits calcineurin phosphatase, does not completely inhibit T cell effector functions. Second, IL-2 deficient mice are immunocompetent in rejecting allografts. Third, the critical role of IL-7 and IL-15 versus IL-2, in regulating T cell growth, has been underscored by *in vivo* studies of T cell functions.

A key role for IL-2 however has emerged in the unraveling of immune regulation. Cytokines, in addition to inducing T cell growth, play vital roles in T cell survival. Central to this finding was the IL-2 deficient mice had an abnormal lymphoproliferative disorder, caused by lack of activation-induced programmed cell death (AICD). T cells in these mice, escaped Fas-induced cell death. Among the γC-chain receptor family cytokines, this function has been restricted to IL-2. Thus, early in the immune response, IL-2 has a primary role to prime T cells to undergo cell proliferation (and survival from AICD). In contrast, in late phases of the immune response IL-2 promotes cell death, which is critical in maintaining immune homoeostasis. These observations underscore the complex interplay in the cytokine networks. Despite these complexities, key observations using genetic studies have delineated the molecular mechanisms of commitment to cytokine secretion profiles by T cells.

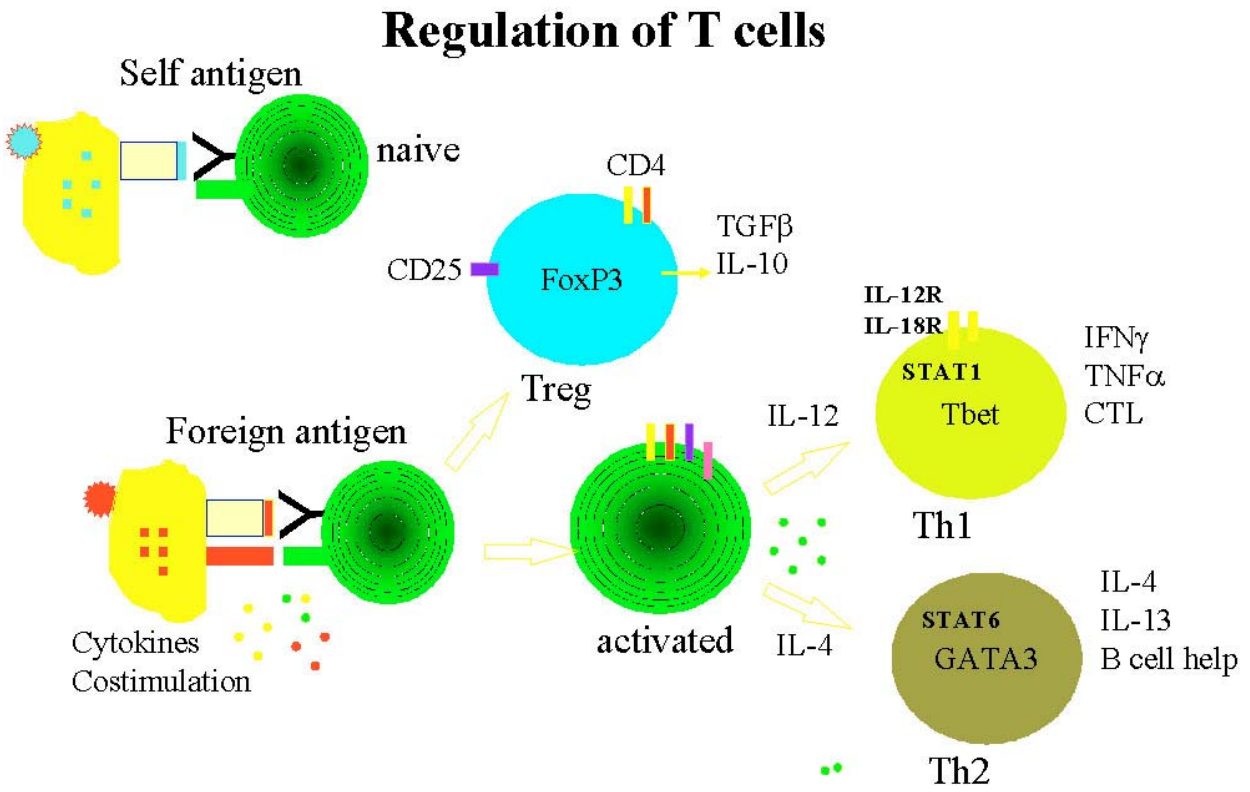

Figure 9. Regulation of T cells. "Self" antigen presented by ACP do not activate T cells (that escape thymic selection), since inflammatory danger signals are not induced. "Foreign" antigens induce inflammatory signals (by upreglation of cell surface molecules and cytokines), which in turn induce effective sustained activation of T cells. A naïve T precursor T cells under the influence of of IL-12 differentiates to express Tbet, and secrete IFNgamma; Th2 cells express GATA3 and secrete IL-4. Th1 cells induce cell mediated immunity, e.g. induction of cytotoxic T cells (CTL), while Th2 cells induce humoral immunity, e.g. secrete cytokines and upregulate cell surface molecules to help in B cell differentiation. Upon appropriate stimulatory conditions, (e.g. CD3+CD46 stimulation) T cells can differentiate into regulatory T cells (Treg). These cells have recently been shown to play significant roles in regulating immune responses.

3.2.3.2. Regulation of cytokine secretion

Advances in the current understanding by which uncommitted T helper (Th) cells develop into mature Th1 and Th2 cells is reviewed (33, 34). Naïve T cells do not secrete cytokines, and undergo dramatic chromatin remodeling to secrete cytokines. In this respect, study of transcription factors in these cell types has been key to revealing the pathways for T cell differentiation. The critical importance of activation of T-bet (T-Box expressed in T cells) in IFNgamma secretion and induction of cell mediated immune responses *in vivo* has been underscored by the susceptibility of T-bet deficient mice to intracellular pathogens, and predisposition of allergic inflammatory diseases (Finotto, Neurath *et al*. 2002; Hatton and Weaver 2003). The signal transduction molecule, STAT1, is required for expression of T-bet. In mature T cells, IFNgamma secretion can occur through two distinct pathways – by signaling through TCR and IL-12/IL-18 cytokine. While TCR-induced IFNgamma secretion is cyclosporin A sensitive, IL-12/IL-18 mediates IFNgamma in a cyclosporin A resistant manner. The mechanism of IL-12/IL-18 induced IFNgamma secretion involves activation of transcription factors GADD (growth arrest and DNA Damage inducible factors)(35) . Thus, the induction of IFNgamma secretion during an acute response e.g during activation through vaccination may be first mediated by TCR stimulation, followed by sustained maintenance of the Th1 phenotype by IL-12/IL-18 signaling, e.g during the persistence phase of the immune response. This understanding is important in development of vaccines (and adjuvants) against intracellular pathogens, such as HIV, malaria and tuberculosis.

The key requirement of STAT-6 in the induction of Th2 responses was shown in STAT6 deficient mice. Naïve T cells express low levels of transcription factor GATA-3, which can be upregulated upon activation. GATA-3 has a strong influence in chromatin remodeling of the IL-4, IL-5 and IL-13 promoter elements (36). Figure 9 shows the schematic representation of the T cell differentiation process for Th1 and Th2 type cells.

The nature of signals during innate immune response has been shown to regulate T helper cell development. A pathogen which induces activation of the

STAT1 alongwith TCR signals, results in activation of Tbet in naïve T cells. This transcription factor induces chromatin modelling of the IFNgamma gene locus and primes T cells to IL-12-mediated signals by upregulating IL-2 receptor β and IL-18 receptor expression. IL-12 and IL-18 can amplify the Th1-type responses. Alternatively, pathogens (such as nematodes) induce inhibitory Th2-type cytokine responses. Naïve T cell which have basal levels, of GATA3 can be induced to secrete low IL-4 leading to Th2 cell development. Thus, priming of the immune system by antigen with appropriate (co-stimuli/innate signals) can strongly influence the ability to obtain the desired results. These signals can be manipulated by developing novel compounds which activate specific pathways of Tbet versus GATA3 activation pathways. Induction of Th1 responses have been implicated in inducing protective immune responses against intracellular pathogens and tumors. On the other hand, modifying responses to Th2-type cytokines has been implicated as strategies to skew away from detrimental Th1 responses in pathologies such as allergies, and autoimmune diseases. Further studies in lineage commitment of Th1 and Th2 cells will be highlighted in gene array studies, recently under intense investigation (37-39).

3.2.3.3. Regulatory T cells

In addition to induction of Th1 and Th2 T cell differentiation, a subset of T cells, which have negative influences on immune responses, and control of autoimmune functions has been described for several years. These cells have been recently termed "regulatory T cells [Treg], or Th3 cells."(40-42). These cells express the IL-2Rα chain at resting state, CD4+CD25+ and can suppress proliferation of naïve T cells. In adoptive transfer models, these cells have been shown to suppress multi-organ autoimmune disease. These cells also express CD38, CD62L and CD103. It is still unclear whether they are biased towards recognition of a subset of self-antigens, since they exhibit diverse TCR usage. Two subsets of Treg cells have been proposed – one naturally occurring, which develop during the normal process of T cell maturation in the thymus, and the second adaptive Treg cells, which develop as a consequence of CD3 and complement receptor and CD46 in the presence of IL-2 induces Treg. The mechanisms by which these Treg cells mediate suppression include secretion of IL-10 and Transforming grown factor (TGF)-β, and constitutive expression of CD25, CTLA4 and Glucocorticoid-Induced [Tumor necrosis Factor] TNF receptor family related gene (GITR); although these cytokines are not necessarily required for the Treg function. The transcription factor, FoxP3, has been identified as being essential for development and function of CD4+CD25+ Treg cells (43). Interestingly, individuals with a mutation in FoxP3 manifest spontaneous autoimmunity (44).

During the process in development of vaccines for cell mediated immune responses, it will be important to study the functions of Treg cells in regulating immune responses. The nature of the activation of this population of Treg cells can potentially have significant impact of the efficacy of vaccine, both in early acute responses, as well as maintenance of sustained memory responses.

3.3. T cell memory

The central objective of vaccine development is the ability to induce long term memory (45-48). Characterization of cell surface molecules has defined memory T cell populations. Depending on the nature of the antigens, memory T cells express varying levels of activation molecules, CD69, CD25, CD27, chemokine receptors, CCR2, CCR5, CCR7, secrete an array of cytokines, and express functional molecules such as perforins and granzymes (49, 50). Several studies have now established that long lived memory T cells survive in the absence of antigenic stimulation, and the proliferative potential of these cells is maintained by cytokines such as IL-7 and IL-15 (51, 52).

Induction of long term immunological protection against pathogens requires high quality as well as quantity of T cells; the threshold of protection is most likely dependent on both components. The initial burst size of T cells, following antigenic exposure leads to the size of the memory cell pool. This concept however, is a challenge for vaccine design, since a large antigenic dose may have detrimental inflammatory effects. Ahmed and colleagues (53) have proposed the programmed model for T cell memory that predicts that the memory cell pool is generated immediately following antigenic stimulation, and thus may not be dependent on the size of the initial effector response. Booster doses with vaccines can enhance the quality of the T cell response, by skewing the memory cell populations to higher affinity clones. The eventual definition of the surrogate marker for measuring protective immune responses will have to compare T cell functions and numbers with clinical efficacy. Several clinical trials in HIV vaccine trials, currently in progress in different parts of the globe, will provide exciting information of T cell functions for the first time.

3.4. Activation and regulation of B cells

3.4.1. T cell dependent B cell responses

The ordered cascade of events leading to B cell responses with help from CD4+ T cells occurs in four successive phases (54, 55). The induction of B cell activation immune response is depicted in Figures 3 and 7.

3.4.1.1 Phase I: *Antigen processing*

Antigen is transported from local sites to T cell zones of the secondary lymphoid organs, where antigen specific CD4+ T helper cells recognize specific antigen. This recognition and subsequent stimulation leads to their activation, expansion and differentiation. The mechanisms of antigen processing and presentation, followed by initial activation of T cells have been described above. The functional consequence of the initial activation of T cells is critical for subsequent B cell activation. The strength and duration of T cell responses influence the effector cell function (56). Cytokines secreted by differentiating T cells, and expression of cell surface molecules regulate B cell activation and differentiation. The central role of CD40L – CD40 interactions has been expanded by several ligand-

Table 1. List of cell surface receptors which mediate signals through interactions with their ligands on pathogens

Receptors	**Ligands**
CD14, LBP	LPS
β2-integrin (CD11/CD18)	
C-type lectins	
Mannose R	Zymosan (yeast), Mannans and Mannoproteins
Complement R (CR1/CD35)	Microbial carbohydrates
Complement R (CR2/CD21)	Antigen+antibody complexes
TLR1	Tri-acyl lipopetides (mycobacteria)
TLR2	Peptidoglycan (Gram positive bacteria) Lipoarabinomannan (mycobacteria) Modulin (Staphyloccous epidermidis) Heat shock protein (host)
TLR3	Double stranded RNA (virus)
TRL4	LPS (gram negative bacteria) Taxol (plant) Fibrinogen (host)
TRL5	Flagellin (bacteria)
TLR6	Di-acyl lipopeptides (mycoplasma)
TLR7	Bropirimine (synthetic) Imidazoquinoline (synthetic)
TLR8	
TLR9	CpG DNA (bacteria)
TLR10	
TLR11	Bacteria in bladder and kidney (64)

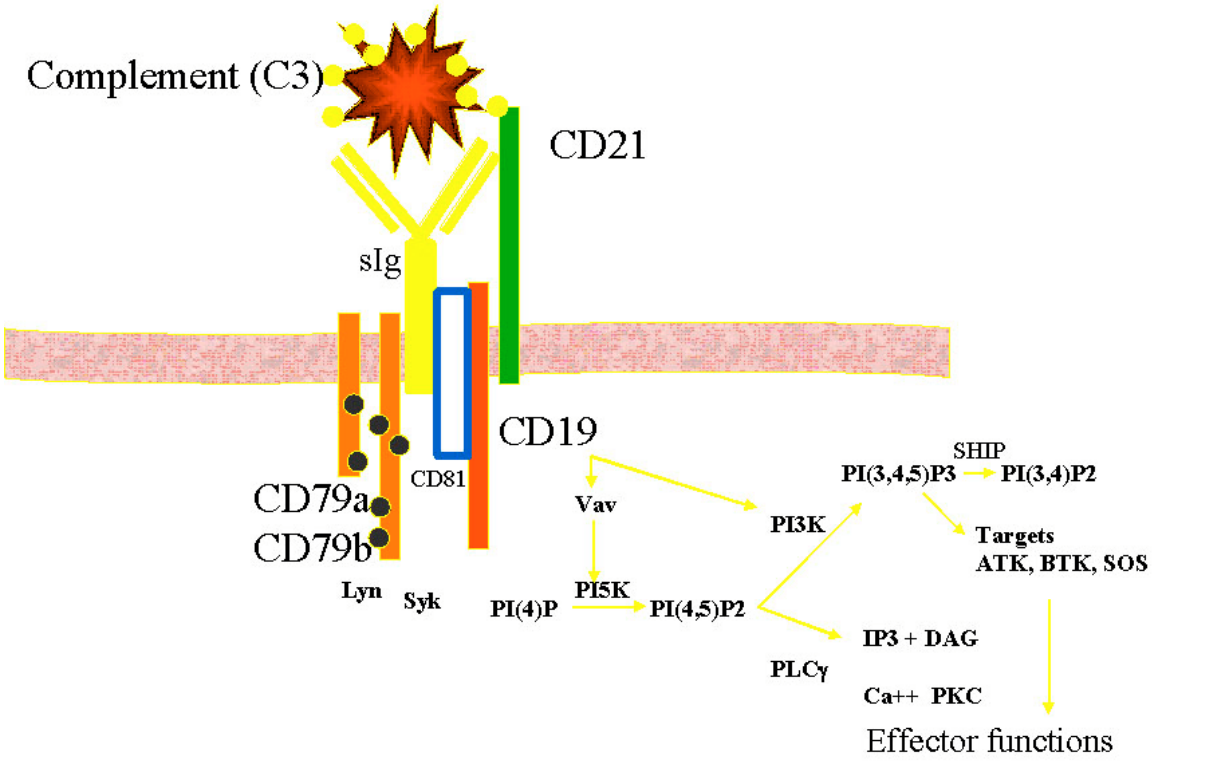

Figure 10. B cell receptor mediated signals. Binding of antigen to the surface immunoglobulin (sIg) results in recruitment of accessory signaling molecules, CD79a, CD79b, CD81, CD19 and CD21. Components of the complement molecules (e.g. C3), which are coated on antigens, bing to their receptors, e.g. CD21, and enhance the aggregation of molecules on the B cell surface. This process results in activation of tyrosine kinases, such as lyn, syk, which leads to recruitment of downstream Phosphatidyl inositol (PI) pathways. In addition activation of phospholipase C (PLC) results in release of inositol phosphate (IPs) and diacyl glycerol (DAG), which results in activation of protein kinase C, and release of intracellular calcium (Ca++). Activation of these second messengers culminates in activation of transcription factors, which ultimately regulate expression of genes, such as ATK, BTK, SOS, and finally immunoglobulin secretion.

receptor pairs, which play roles in qualitative and quantitative nature of the B cell response. In this respect kinetics of proliferation, maintenance of antibody secretion, strength of signal, somatic mutation, isotype-switching and B cell apoptosis are finely regulated by T cell help.

3.4.1.2. Phase II: *B cell activation*

This phase of the B cell response is initiated by recognition of antigen by the antigen-specific B cell receptor (surface immunoglobulin). Similar to T cells, B cells also develop a synapse with APC. Signals delivered to B cells through the antigen receptor activate B cells to upregulate receptors, priming them to be activated further by signals from T helper cells. The nature of these signals through the cytoplasmic tails of CD79a, CD79b, CD19, CD21 and CD81 cell surface molecules, involving tyrosine phosphorylation of kinases and adaptor molecules, results in increase in intracellular calcium, and activation of transcription factors (Figure 10). This phase of the B cell response occurs at the border of follicles in the secondary lymphoid tissue. Here naïve antigen-specific B cells differentiate into precursors of differentiated plasma cells and memory cells.

3.4.1.3. Phase III: t*he germinal center reaction*

After a week of antigen exposure, the lymphoid follicle polarizes into T cell zones, B cell proliferating zones (centroblasts) and non-cycling B cells (centrocytes). In the germinal center, centrocytes undergo somatic hyper-mutation, and are positively selected as high affinity clones to the original antigen. These cells exit the germinal center as effector cells (antibody secreting cells) and traffic to various local sites. In the germinal center, B cells also undergo somatic hypermutation and RNA editing, by which affinity maturation occurs, allowing for switching to higher affinity antibodies. Isotype class switching also occurs in the germinal centers, which is required for diversity of antibody functional responses (e.g. neutralizing functions, antibody dependent cellular cytotoxicity, complement fixing etc). Recently the molecular mechanism for somatic mutation, class switch hypermutation and RNA editing has been shown to involve the aminodeaminase enzyme. The critical requirement of this enzyme for immunoglobulin diversity has been confirmed by lack of somatic mutation and immunoglobulin class switching in humans with a primary immune deficiency with a defect in the AID enzyme gene. Here again, understanding the molecular mechanisms by which antibody diversity and differentiation to induce appropriate functions will be critical in development of vaccines and biologics against pathogens.

3.4.1.4. Phase IV: m*emory*

Immune memory is characterized by rapid response rates to antibody secretion. Peak antibody responses following activation of memory B cells occurs within 3-4 days of antigenic stimulation. During this response, memory B cells differentiate into plasma cells. The memory B cell compartments comprise of i) long-lived mature plasma cells which predominantly reside in the bone marrow and ii) small memory B cells, which do spontaneously secrete antibody, but differentiate rapidly into high affinity antibody secreting cells upon antigenic stimulation. (57).

3.4.2. T cell independent B cell responses

B cells can be activated by antigens that are expressed on the surface of pathogens, in a highly repetitive form by cross-linking with the antigen receptor (58-61). B cells respond multivalent antigens in the absence of MHC-restricted T cell help. In this respect, polysaccharide antigens, lipopolysaccharides, Ig, DNA membrane proteins can activate the other subset of B cells (B1 cells) in a T-independent manner. B1 cells express high levels of IgM, home to peritoneal cavities and gut-associated lymphatic tissues (GALT). Splenic marginal zone B cells, located in the margins of white and red pulp, are also a distinct B cell population that has been shown to play a critical role in the T cell independent B cell response. Mice deficient in the tyrosine kinase pyk2, have no marginal zone B cells, and fail to secrete IgM, IgG3 and IgG2a in response to Ficoll, a T cell independent antigen (62). T-independent B cells obtain help from non-T cells. In this respect, BLys/BAFF (B-lymphocyte stimulator/B cell activating factor) and APRIL (A proliferation inducing ligand) expressed on macrophages and dendritic cells, bind to its receptors on B cells (TAC1 [Transmembrane activator and CAML interactor] and BCMA [B cell maturation antigen]) on cells and deliver signals for B cell differentiation (63).

Carbohydrate antigens have been utilized to make successful vaccines against *Streptococus pneumonia* and *Haemophilus influenza*. It has been well established that Capsular polysaccharides of these pathogens induce a T-independent immune response characterized by the predominantly IgM responses, markedly reduced isotype switching no memory response, and are not immunogenic in infants. In this respect two types of T-independent antigens TI-1 and TI-2, have been described. T-independent (TI)-1 antigens such as lipopolysaccharide (LPS) from the outer membrane of the gram-negative cell wall and bacterial nucleic acid, activate B-lymphocytes by binding to their specific Toll-Like Receptors rather than to B-cell receptors. Antibodies generated against TI-1 antigens are natural antibodies since they are directed against bacterial antigens present in the body. TI-2 antigens, such as capsular polysaccharides, are molecules with multiple, repeating subunits. These repeating subunits activate B-lymphocytes by simultaneously cross-linking a number of B-cell receptors. The inability of TI antigens to induce long term memory responses, has led to the development of conjugate vaccines, which induce activation of T cell help, resulting in Ig class switching, and induction of long term memory to the carbohydrate antigens. Development of vaccines against T-independent antigens is important since, several infectious pathogens have highly repetitive antigen determinants in their envelope. In cases of pathogens which elicit acute inflammatory responses, an early efficient neutralizing IgM response can be critical for survival of the host.

4. PERSPECTIVE

The review gives a brief overview of the recent advances (2003-2004) in immunological concepts that

have helped understand the generation of an effector and memory immune response. With the advent of human genome sequence, and data mining methodologies, there will be an explosion of information in various aspects of immunology. Ultimately, understanding the immune responses will lead to development of more effective, targeted, specific and safe prophylactic and therapeutic vaccines for infectious diseases, chronic illness, and cancer.

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