Review of Literature
1. Overview:

The first reaction of the immune system when it encounters an antigen is an inflammatory response in which cells responsible for the host defense rush to the site of inflammation and secrete cytokines and chemokines. Amongst these are the cells that represent the innate immune system and have the ability to produce various cytokines. These cells and cytokines have potent antimicrobial activity but they are not pathogen specific. Instead, they recognize molecular patterns that are conserved and shared by a large number of microorganisms. Although these innate immune cells are efficient in preventing an infection and greatly reduce the microbial load, total cure of the infection is achieved by the adaptive immune system. The adaptive immune system is antigen specific and comprises of T cells and B cells, the former conferring cell mediated and the later humoral immunity to the host. T cells and B cells have the ability of clonal expansion of an antigen specific cell and retain memory of the antigen encountered. The innate and adaptive immune systems are not just complimentary mechanisms of host defense but regulate each other through cell to cell contact or through secretion of various cytokines. Especially the cytokine milieu during the inflammatory response provides the necessary platform for the migration of the antigen specific T cells to the lymph nodes where they meet the APCs. T cells then take over the host defense and, through cytokine secretion and other effector mechanisms, tightly regulate the immune response, and bring about effective removal of infectious agents.
2. Classification of Cytokines and Cytokine Receptors:

Cytokines are small, soluble protein molecules secreted by many cells including the cells of the immune system and can alter the function of the same cell that produces it, in an autocrine fashion or exert their effect on other cells, in a paracrine manner. According to their similarity in structure, the cytokines are grouped into three different families- the haematopoietins, interferons, and TNF family members- their receptors also grouped likewise. Most of the cytokines secreted by the T cells, fall into the haematopoietin group. Many of these cytokines are similar in function and genetically closely related such as IL3, IL4, IL5, IL13 etc. Moreover they bind to closely related receptors which are classified as class I cytokine receptors. For instance, IL2, IL4, IL7, IL9, IL15 and IL21 have been known to share the same γ chain (Boulay, Shea & Paul, 2003). IFN-γ, also a T cell cytokine, does not share similar receptors as the haematopoietins, but IFN-γR is a member of a small family of cytokine receptors that includes receptors for IFN-α, IFN-β and IL10 (Renauld, 2003). Members of the TNF family members form functional trimers that are mostly membrane bound. TNF family members involved in T cell effector functions include TNF-α, TNF-β, Fas ligand and CD40 ligand. The receptors for this cytokine family form the TNFR family of receptors. TNFR I and II have the ability to interact with both TNF-α and TNF-β, whereas Fas ligand and CD40 ligand bind to Fas and CD40 expressed on the target cells. Most of the TNFR family members contain death domains in their cytoplasmic tails and can activate a cascade of cellular caspases leading to apoptotic death. Mice and humans having mutations in Fas and Fas ligand genes develop
lymphoproliferative disorders, underscoring an important role for Fas in lymphocyte homeostasis (Hehlgans & Pfeffer, 2005).

2.1 Common γ Chain Receptor Family:

This family of cytokine receptors has a gamma chain (CD132) as one of its receptor subunits, which is common to all. The cytokines that share this common γ chain use a similar downstream signalling pathway and are vital for T cell development and function. From thymic selection to effector phases of the immune response and even memory T cell generation and maintenance, these cytokines play an important role as growth and survival factors (Malek, Porter & He, 1999). γ chain dependent signalling is evidently a crucial factor in thymocyte maturation and IL7, a γ chain dependent cytokine, has been reported to be a major regulator of thymocyte development. Analyses of thymocyte development in γ chain deficient, IL7 deficient, or IL7 receptor α (IL7Rα) chain deficient mice have revealed diminished numbers of thymocytes and low expression of anti-apoptotic protein Bcl2 (Peschon et al., 1994, von Freeden Jeffry et al., 1995, von Freeden Jeffry, 1997). Bcl2 expression in the transition from double negative (DN) CD44^-CD25^- to DN CD44^-CD25^+ and from double positive (DP) TCR^{\beta} to DP TCR^{int} contributes to normal cellularity of the thymus (Nakajima & Leonard, 1999). Apart from induction of Bcl2, IL7R mediated signalling also promotes expansion and survival of thymocytes through the phosphatidylinositol 3-kinase (PI-3 kinase)–protein kinaseB (PKB) pathway and regulates their differentiation through Stat5 (Pallard et al., 1999). γ chain deficient and IL7R deficient mice lack γδ T cells suggesting a distinct role of
IL7 mediated signalling in the TCR rearrangement of these cells (Malek, Porter & He, 1999). γ chain mediated signals also play a role in B cell development as γ chain and IL7R deficient mice show impaired B cell development (Peschon et al., 1994, Maraskovsky et al., 1996). Other γ chain dependent cytokines, IL2, IL4, and IL9, however do not affect early lymphocyte development. Nevertheless, gross defects in T cell homeostasis in the peripheral compartments of IL2 deficient mice leads to age related lymphoproliferative disorders and autoimmune pathologies (Sadlack et al., 1993, Kramer et al., 1995). IL15 and IL21 are two other cytokines of this family and have effect on NK cell and B cells (Suzuki et al., 1997, Vosshenrich & Di Santo, 2001).

The effects of γ chain dependent cytokines in the peripheral T cell compartments depend upon the differential upregulation of receptor expression. Naive T cells express IL7R, IL15Rα, IL2Rβ and γ chain but do not express IL2Rα (CD25) (Schluns et al., 2000, Budagian et al., 2006, Kim, Imbert & Leonard, 2006). IL7 has been reported to be important for naive T cell survival and is vital for homeostatic proliferation of naive CD4 and CD8 T cells in immunocompromised hosts (Vella et al., 1997, Schluns et al., 2000). IL15 seems to be important for CD8 T cell survival and production as IL15Rα and IL15 deficient mice have approximately half the normal number of naive CD8 T cells (Lodolce et al., 1998, Kennedy et al., 2000). IL7 and IL15 are crucial for the maintenance of memory CD8 T cells. As already discussed, IL7 is a survival factor and memory CD8 T cells are known to express high levels of IL7Rα rendering this cytokine central to the maintenance and survival of memory CD8 T cells (Schluns et al., 2000, Goldrath et al., 2002). IL15 on the other hand is important for the proliferation rather than survival of memory CD8 T cells.
Administration of anti-IL2/IL15Rβ inhibits the proliferation of memory CD8 T cells \textit{in vivo} (Ku et al., 2000). Thus, it may be possible that IL15 and IL7 act in synergy to maintain the CD8 memory pool.

\textbf{2.2 Class II Cytokine Receptor Family:}

The major antiviral factors and modulators of inflammatory responses belong to this family and signal through heterodimeric receptor complexes that comprise the class II cytokine receptor family (Renauld, 2003). It consists of 12 receptors, eleven of them combine as various heterodimers and transduce signals for 27 cytokines. These cytokines can be grouped into four different categories based on their structural similarities. There are six members in the IL10 family, 17 type I interferons, 1 type II interferon and 3 IFNλs. One of the receptors of class II cytokine receptor family, IL22 binding protein, is a soluble receptor and is a natural antagonist of one of the IL10 family members, IL22 (Kotenko & Langer, 2004). The cytokines using this family of receptors for signalling are involved in various functions of induction and regulation of the immune response. Type I and II interferons as well as IFNλs are well known for their antiviral properties (Takaoka & Yanai, 2006). IFNs stimulate expression of many cell surface molecules such as MHC I and induce and activate many pro-apoptotic genes such as TRAIL, caspases, Bak and Bax (Singer & Maguire, 1990, Clemens, 2003). They also repress anti-apoptotic proteins like Bcl2 and inhibitor of apoptosis protein (IAP) potently (Clemens, 2003). IFN-γ, however, has other indispensable functions as an inducer of cell mediated immunity that are critical for protection of the host from intracellular parasites (Shtrichman & Samuel,
2001). It is significant for T cell differentiation and mediates anti-tumor and cytotoxic responses (Murphy and Reiner, 2002, Dunn, Old & Schreiber, 2004). Though IL10 family members share homologous receptors, they are more prominent in regulating the inflammatory and T cell responses (Renauld, 2003). IL10 itself has been recognized as a key modulator of the immune response and is principally secreted by T cells with regulatory activities. It potently inhibits the production of proinflammatory cytokines such as IFN-γ, TNF and IL1 (Moore et al., 2001).

2.3 TNF/TNF Receptor Family:

Majority of the receptors and ligands of this family are expressed by immune cells and affect various responses such as proliferation, survival, differentiation and apoptosis of the responding cells. The ligands are type II transmembrane proteins (intracellular N terminus) that are biologically active as self-assembling noncovalent trimers. The 25-30% homology in the amino acid sequence of the internal aromatic residues of the interacting proteins allows them to form trimers (Fesik, 2000). However, the external surface of these ligands bears little similarity, which accounts for the receptor specificity of these ligands. Some of these ligands, eg., TNF exist in two forms, as a membrane bound ‘pro’ and a proteolytically cleaved soluble ‘mature’ form. Both these forms are biologically active (Idriss & Naismith, 2000). The TNFR-like receptors are type I transmembrane proteins which form elongated structures through disulfide bonds in the cysteine rich domains (CRD). These CRDs are 40 amino acid pseudorepeats are defined by 3 intrachain disulfide bonds between 6 highly conserved cysteines, though the number of CRDs in different receptors may
CRDs are hallmark of the TNFR superfamily (Smith, Farrah & Goodwin, 1994).

The TNF/TNFR family members utilize two unique modes of signalling. This involves two principal classes of cytoplasmic adaptor proteins: death domain (DD) molecules or the TNF receptor associated factors (TRAFs) (Chinnaiyan et al., 1996, Kischkel et al., 2000, Inoue et al., 2000). The choice of the adaptor molecules depends on whether the cytoplasmic tail of these receptors contains a death domain or TRAF interacting motif (TIM) (Hehlgans & Pfeffer, 2005). The death domain containing group comprises of Fas (CD95), TNFRI, TNF related apoptosis inducing ligand receptor 1 (TRAIL-R1) (DR4), TRAIL-R2 (DR5), TRAIL-R4 (DcR2), TNF-like receptor apoptosis mediating protein (TRAMP) (DR3), ectodysplasin (EDA) receptor (EDAR) which, on activation, recruit adaptors like Fas-associated death domain or TNFR associated death domain (TRADD) (Hehlgans & Pfeffer, 2005). These molecules are capable of activating the caspases and subsequently lead to apoptosis (Scaffidi et al., 1999).

The second group includes TNFRII, CD27, CD 30, CD40, OX40, 4-1BB, LTβR, B cell activating factor receptor (BAFFR), B-cell maturation antigen (BCMA), receptor activator of NF-κB (RANK), transmembrane activator and calcium-signal modulating cyclophilin ligand (CAML) interactor (TACI), Fn14, herpes virus entry mediator (HVEM), activation induced TNF-receptor (AILTR), X-linked EDA-A2 receptor (XEDAR) and the member of the TNFR family (TROY) which recruit TRAFs (Hehlgans & Pfeffer, 2005). There are six TRAFs recognized in the mammalian system (TRAF1 to TRAF6), all of which have a highly conserved TRAF domain (Grech et al., 2000). These TRAFs have no
enzymatic activity, nevertheless, they can induce many signalling events that can have consequences ranging from cell proliferation, death and differentiation.

Functions of the TNF family members vary from principal regulators of lymphoid organogenesis and maintenance of lymphoid microarchitecture to host defense where they play roles in inflammation, sepsis and impart protection against various pathogens. TNFR1 deficient mice are unable to control the replication of *Listeria monocytogenes* in the phagocytes though other microbicidal mechanisms like the production of reactive oxygen and nitrogen intermediates are intact in these mice (Endres et al., 1997). Similarly, when these mice are infected with *Leishmania major* or *Trypanosoma cruzi*, higher parasite burden and mortality than the control mice is observed (Nashleanas, Kanaly & Scott, 1998, Castanos-Velez et al., 1998). Using *Mycobacterium tuberculosis* infection model, it has been suggested that TNF augments the phagocytic and killing ability of macrophages working in concert with IFN-γ (Bekker et al., 2001). TNF family members are reported to play a critical role in inflammatory bowel disease (IBD) (Neurath et al., 1997, Kojouharoff et al., 1997, Stopfer et al., 2004). TNF also has a role to play in systemic endotoxic activity leading to fever, hypotension and shock (Rothe et al., 1993, Pfeffer et al., 1993).

3. Cytokines and Modulation of T Cell Responses:

The immune system needs to distinguish between the types of pathogens it encounters and then employ an effective clearance mechanism. Participation of T cells is essential for proper removal of the pathogens. Cytokines, apart from their role in T cell development, memory generation and maintenance, play a
vital role in the effector phases of the immune response by modulating T cell responses and their differentiation. From antigen presentation to T cells by the APCs until the effective removal of the antigen the T cell responses are synchronized by the cytokine milieu generated in response to the type of antigen. CD4 T cells facilitate the distinction between various pathogens and recruit specific clearance mechanisms. Striking consequences are observed in CD4 T cell compartment as their differentiation and effector activity are orchestrated by the cytokine milieu at every phase of the immune response.

### 3.1 Th1 / Th2 Differentiation and Cytokine Secretion:

Naive T helper cell activation by the APCs initiates a differentiation programme that depends on (1) the nature of the pathogen encountered, (2) the antigenic dose, (3) cytokine signalling, (4) action of the costimulatory molecules and (5) induction of key transcription factors (Carballido et al., 1997, Ausubel, Krieger & Hafler, 1997, Murphy et al., 2000, Farrar, Asnagli & Murphy, 2002). These armed effector T helper cells delineate into either of the two functional groups, which are segregated based on their cytokine secretion pattern. The two forms that a T helper cell post-activation may diverge to are categorized as Th1 or Th2 cells (Murphy & Reiner, 2002). Th1 cells predominantly produce IL2 and IFN-γ upon activation whereas Th2 cells are characteristic in the production of IL4, IL5 and IL13 (Murphy & Reiner, 2002). The production of these cytokines by the T helper cells leads to differential immune responses and helps in effective clearance of the pathogen. Th1 cells are reported to be involved in cell-mediated immunity helpful in the clearance of intracellular pathogens and delayed type hypersensitivity (DTH) responses. Th2 cells, on the other hand promote allergic
responses that are critical for the elimination of helminths and other extracellular parasites (Abbas, Murphy & Sher, 1997). The help imparted to B cells by these two different cell types, in context of heavy chain class switching, is completely different and cross regulatory. Th1 cytokine IFN-γ encourages the switching to IgG2a and IgG3 (Severinson, Fernandez & Stavnezer, 1990, Snapper et al., 1992, Collins & Dunnick, 1993, Huang et al., 1993), while Th2 cytokine IL4 favours the switching to IgG1 and IgE (Vitetta et al., 1985, Coffman et al., 1986, Gascan et al., 1991). IFN-γ can potently antagonize the IL4 driven class switching to IgG1 and IgE, whereas IL4 can inhibit the class switching to IgG2a and IgG3 (Berton & Linehan, 1995, Shimoda et al., 1996, Takeda et al., 1996).

Another feature of these two groups of cytokines is that they serve as autocrine growth factors and favour the differentiation of naive T helper cells to that subset. They antagonize the function of the other group effectively and thus the T helper cells progressively polarize in one direction (Boehm et al., 1997, Nelms et al., 1999). This process of differentiation of naive T cell precursor is influenced by the cytokine milieu at the time of TCR engagement by appropriate MHC-peptide complex.

The differentiation of naive T helper cells to Th1 or Th2 is regulated by many cytokines, which, in the case of Th1 differentiation is much clear. The innate immune components, which are the primary responders to any infection such as the DCs and macrophages, and act as APCs are the main source of cytokines that promote Th1 differentiation. These APCs secrete IL12, the main cytokine implicated in Th1 differentiation, IL23, IL27, IFN-γ, and the type I IFNs (Agnello et al., 2003). IL12 drives the naive T helper T cells to proliferate and induces production of IFN-γ by NK cells and T cells (Kobayashi et al., 1989,
Hsieh et al., 1993, Manetti et al., 1993). IL12 further promotes the differentiation of Th1 phenotype by acting on APCs to produce IFN-γ and more IL12 (Grohmann et al., 1998, Frucht et al., 2001, Grohmann et al., 2001). T cells, NK cells, and APCs express IL12 receptor (IL12R) through which IL12 signals to promote cell-mediated immunity. Th1 cells express IL12R subsequent to activation by the APCs. The IL12R comprises of two subunits β1 and β2, and mice deficient in either of the subunits show deficient Th1 responses, suggesting the critical role of this cytokine in Th1 differentiation (Chua et al., 1995, Presky et al., 1996, Magram et al., 1996, Wu et al., 1997, Wu et al., 2000, O'Shea & Paul, 2002). IL12 receptor is not expressed by resting T cells, but NK cells show low levels of IL12R expression. This explains the ability of NK cells to react rapidly to IL12. T cells upregulate IL12R expression upon TCR engagement. This upregulation is further enhanced by IL12 itself, IFN-α, IFN-γ, TNF and costimulatory signals through CD28 (Szabo et al., 1997, Rogge et al., 1997). Although the importance of IL12 in Th1 development has been demonstrated using gene deficient mice, it does not seem to be the absolute requirement for Th1 differentiation. Two other cytokines that are homologous to IL12, IL23 and IL27, also can induce the production of IFN-γ by T cells and dendritic cells and help in Th1 polarization, either independently or in synergy with IL12 (Oppmann et al., 2000, Chen et al., 2000, Yoshida et al., 2001, Belladonna et al., 2002). Mice deficient in either chains of the IL12Rβ allowed to study the significance of IL12 in promoting cell mediated immunity. It was then confirmed that a deficiency in IL12Rβ2 (p35 chain) leads to a greater susceptibility of these mice to EAE as compared to mice deficient in IL12Rβ1 (p40 chain) (Becher, Durell & Noelle, 2002). This IL12Rβ2 is shared by another
cytokine IL23 that seems to play role in autoimmune diseases (Cooper et al., 2002, Becher, Durell & Noelle, 2002). IFNγ induction by IL12 is further enhanced by an unrelated cytokine IL18, both these cytokines inducing the expression of receptors for each other (Yoshimoto et al., 1998, Lawless et al., 2000, Sareneva, Julkunen & Matikainen, 2000, Smeltz et al., 2001, Frucht et al., 2001). However, IL18 alone is unable to direct a Th1 response, and in the absence of IL12 can even stimulate Th2 responses (Nakanishi et al., 2001). In contrast to its functions to promote Th1 differentiation, IL12 with IFNγ strongly suppresses the differentiation of naive T helper cells to Th2 and the production of Th2 cytokines (Trinchieri, 2003). Another major regulator of Th1 responses is IFN-γ itself. Apart from being the signature cytokine of Th1 responses IFN-γ is also essential for Th1 stabilization. IFN-γ provides a positive feedback loop promoting the production of IL12 by the APCs and expression of IL12Rβ2 on the CD4 T cells (Ma et al., 1996, Afkarian et al., 2002). Certain transcription factors have been reported to be crucial for the action of these cytokines in mediating Th1 polarization. IL12 signalling through signal transducer and activator of transcription 4 (STAT4) and induction of T-box protein expressed in T cells (T-bet) expression by IFN-γ potentially regulate the polarization of CD4 T cells to a Th1 phenotype (Murphy & Reiner, 2002).

Although the importance of IL12 in Th1 development has been demonstrated using gene deficient mice, it does not seem to be the absolute requirement for Th1 differentiation. Other factors like toll like receptor (TLR) mediated signalling and induction of characteristic transcription factors induced in Th1 cells are reported to be significant players in Th1 polarization. In an infection model using *Toxoplasma gondii*, repeated infections with a non-lethal dose or
repeated immunization with soluble extract, IL12 de-
response. Although this response is low in magni-
tude from infection, double deficiency of IL12 and Il
indicating that IL10 limits the efficiency of the
MyD88-deficient animals that lack TLR-signalling c
Th1 response (Jankovic et al., 2002). Similarly, indi-
ator T-bet is reported in T cells when IL12 is block-
mice (Mullen et al., 2001). The importance of T-bet
demonstrated by the fact that Th2 cells or CD8 T cell
be are repolarized to secrete IFNγ (Szabo et al.,
cannot induce IFNγ production by Th2 cells that are
which is not expressed in Th2 cells (Heath et al., 2000
rapidly induces T-bet in lymphoid and myeloid
(Lighvani et al., 2001). Thus, existence of an IL12
with signalling through TCR and other recep-
commitment.
The factors regulating initiation of Th2 polarization at
some DC subsets are reported to bias the Th1/Th2 re-
Th2 differentiation are produced by the APCs as co-
human system, thymic stromal lymphopoietin (TSLP
factor that drives Th2 polarization. TSLP is highly ex-
and TSLP activated DCs induce the production of IL
by CD4 T cells (Soumelis et al., 2002). In vitro
generation is usually a response to exogenous IL
activation, however, IL4 transcription has been repo
upon activation (Grogan et al., 2001, Ansel et al., 2004). IL4 receptor (IL4R) is normally expressed on naive T cells and transduces its signal through STAT6 which along with nuclear factor of activated T cells (NFAT), activator protein (AP1) and NFκB induce the transcription of IL4 and other Th2 cytokines (Li-Weber & Krammer, 2003). This also initiates the synthesis of transcription factor GATA3 that is involved in Th2 regulation (Zheng & Flavell, 1997). The production of Th2 cytokines provides an autocrine positive feedback loop that favours Th2 polarization. Th2 cytokines effectively suppress the production of IFNγ and thus abrogate Th1 differentiation (Seder et al., 1992, Hsieh et al., 1992). Although, in vitro studies strongly suggest the presence of IL4 as the driving force for Th2 commitment, the relative scarcity of IL4 in the T cell regions of secondary lymphoid organs where the APCs present the antigen to naive T cells, leaves this process poorly understood in physiological settings. However, new studies using in vitro and in vivo systems provide evidence for IL6, secreted by the APCs, being involved in the induction of IL4 secretion by CD4 T cells at the time of priming (Rincon et al., 1997, Diehl & Rincon, 2002). IL6 also inhibits Th1 polarization by inducing the expression of suppressor of cytokine signalling 1 (SOCS1) in CD4 T cells, thereby inhibiting the production of IFN-γ (Diehl et al., 2000). Apart from cytokine signalling, other factors that affect the Th1/Th2 commitment may be the antigen dose and differences in the TCR mediated signalling. High doses of antigen have been reported to favour Th1 polarization whereas low doses support the commitment of naive CD4 T cells to Th2 phenotype (Constant & Bottomly, 1997). The deficiency of various kinases and molecules involved in TCR mediated signalling also influence Th differentiation (Schaeffer et al., 2001).
3.2 Regulation of T Cell Responses:

T cell mediated suppression of the immune response to self antigens that are not expressed in the thymus and other innocuous antigens is a characteristic feature of peripheral mechanisms of regulation of the immune response. For this purpose, there exists a subset of T cells known as regulatory T cells (Tregs). These Tregs suppress the immune response either by cells to cell contact by expressing certain inhibitory molecules on the cell surface, or through the secretion of immunoregulatory cytokines, IL10 and TGF-β (Shevach, 2006). Because of these specific immune suppression mechanisms, the Tregs render responding classical T cells anergic, thereby attenuating the ongoing immune response. Anergy can be defined as a state of hyporesponsiveness of T cell clones upon rechallenge with fully competent APCs. Induction of anergy in T cells is due to absence of costimulatory signals at the time of T cell priming. Treatment of APCs with IL10 prior to T cell activation or presence of IL10 at the time of T cell priming also induces anergy (Groux et al., 1996, Steinbrink et al., 2002). Although, anergy induced by the absence of costimulation is reversed by addition of exogenous IL2, unresponsiveness of IL10 treated T cells remains irreversible upon IL2 addition, and can only be overcome by neutralizing IL10 (Groux et al., 1996). Depending on the mechanisms of action employed to suppress the immune response the Tregs are classified into several distinct subsets.

3.2.1 Tr1 Cells:

Ovalbumin (OVA) TCR transgenic T cells cultured with OVA in the presence of IL10 result in the generation of a new subset of T cells that suppresses the
proliferation of T cells of same peptide specificity and have a completely different cytokine secretion pattern than that of Th1 or Th2 cells. These cells are known as Tr1 cells and produce IL10 and TGF-β or only IL10. A minimal level or completely absent IL2, IFNγ and IL4 production is observed in these cultures. In addition to this, Tr1 cells fail to proliferate upon polyclonal stimulation by anti-CD3 (Groux et al., 1997). However, their proliferation upon anti-CD3 stimulation can be significantly enhanced by the addition of exogenous IL2. Despite this deficit in proliferation, activation marker profiles of these Tr1 cells are normal as they express normal levels of CD25, CD69 and CD40L subsequent to TCR mediated activation. Resting Tr1 cells express high levels of IL2/IL15Rβ chain and the common γ-chain (Bacchetta et al., 2002). Tr1 cells, by the virtue of producing IL10 are known to affect a variety of cellular response. They suppress naive and memory T cell responses and have been shown to prevent the development of Th1 and Th2 mediated immune pathologies. They can, as well, suppress the immune responses to pathogens, tumor antigens and alloantigens (Cavani et al., 2000, Lecart et al., 2001, Iwashiro et al., 2001, Graca, Cobbold, Waldmann, 2002). Although Tr1 cells need to be activated by a specific antigen to exert their effects, once activated they can act in an antigen non-specific manner (Groux et al., 1997). Moreover, reversal of the Tr1 mediated suppression by neutralizing IL10 suggests that these suppressive effects are conferred by the production of IL10.

3.2.2 Th3 cells:
Oral administration of myelin basic protein (MBP) in mice leads to the generation of a distinct subset of suppressor T cells. These cells secrete TGF-β
and can prevent the induction of experimental autoimmune encephalomyelitis (EAE) in a TGF-β dependent manner (Chen et al., 1994). In humans as well, oral treatment of multiple sclerosis patients with MBP induces enhanced numbers of TGF-β producing T cells (Fukaura et al., 1996). These cells are known as Th3 cells and constitute a distinct subset of regulatory T cells. However, in experimental systems other than for oral tolerance Treg population that exclusively produces TGF-β has not been frequently observed. Nevertheless, these cells may be important in immune subversion by tumors to suppress the generation of tumor specific cytotoxic T cells (CTL), as many tumor cell lines actively produce TGF-β (Zhang et al., 2006).

3.2.3 CD4+CD25+Foxp3+Tregs or Natural Tregs (nTregs):

CD4 T cells that constitutively express CD25 (IL2Rα) can also suppress the immune response effectively. Due to their development in the thymus, they are known as naturally occurring Tregs or nTregs (Shevach, 2002). However, several reports indicate the possibility of peripheral development mechanism of Tregs and have shown that costimulated TCR mediated naive CD4 T cell activation in the presence of TGF-β leads to the generation of CD4+CD25+ cells that show a significant contact dependent suppressor activity mediated by IL10 and TGF-β (Chen et al., 2003, Fantini et al., 2004, Tai et al., 2005, Bettelli et al., 2006). These cells develop relatively late in the thymus as shown by experiments including thymectomy at day 3 after birth, in which case mice develop autoimmunity. On the other hand, adult thymectomy resulted in no such pathologies (Asano et al., 1996). These Tregs are selected for the self peptide-MHC complexes and costimulatory signals for their development, as mice
lacking CD28, CD80 or CD86 show diminished numbers of Tregs (Jordan et al., 2001, Hsieh et al., 2004, Salomon et al., 2000). It is suggested that this deficit in Treg population is due to the combination of direct effect of lack of costimulatory signals for thymocyte development and abrogated IL2 production by non-regulatory T cells in the periphery in the absence of costimulatory interactions. However, Tregs generated in mice deficient in CD28 are functional, thus proving that CD28 is not compulsory for Treg lineage commitment (Salomon et al., 2000, Tai et al., 2005). IL2 signalling through the IL2R has been implicated in the development of Tregs and IL2 and IL2R deficient mice develop lymphoproliferative disorders prominently (Malek & Bayer, 2004). IL2 is required for the in vitro and in vivo activation of Tregs and expression of CD25, the high affinity component of the IL2R. This IL2, necessary for in vivo Treg maintenance, is produced by other T cells, thus conferring a feedback mechanism in which responder T cells produce IL2 for activating Tregs that inhibit the production of IL2. Dependency of natural Tregs on IL2 is a vital feature that distinguishes them from other Treg cells that are dependent on IL10 and TGF-β (Furtado et al., 2002, Thornton et al., 2004, Setoguchi et al., 2005). Conversely, adoptive transfer of CD4 positive T cells from IL2 deficient mice offers protection to experimental allergic encephalomyelitis, rendering the possibility of IL2 independent mechanisms in natural Treg development (Furtado et al., 2002, Curotto de Lafaille, 2004). Another characteristic feature of the naturally occurring Tregs is the expression of Foxp3, a member of the family of forkhead winged helix transcription factors. First identified as a cause for recessive inflammatory disorder in mutant scurfy mice, Foxp3 is expressed in CD4+CD25+ peripheral T cells and CD4+CD25+CD8- thymocytes, whereas
no other T cells, thymocytes, B cells or natural killer cells have been known to express Foxp3 (Brunkow et al., 2001, Bennett et al., 2001, Hori, Nomura & Sakaguchi, 2003, Fontenot, Gavin & Rudensky, 2003). Naive CD4 T cells or cells committed to the Th1 or Th2 lineage are not known to express Foxp3. Foxp3 deficient mice are deficient in CD4+CD25+ T cells and are susceptible to inflammatory disorders as the scurfy mice (Fontenot, Gavin & Rudensky, 2003). Moreover, CD25-CD4+ or CD8 T cells transduced with Foxp3, act efficiently as Tregs and are able to suppress proliferation of other T cells in vitro and prevent the development of inflammatory bowel disease (IBD) in vivo (Hori, Nomura & Sakaguchi, 2003, Fontenot, Gavin & Rudensky, 2003). These transduced cells are unable to produce IL2 but show normal expression of Treg associated molecules. Thus, Foxp3 seems to be the master regulator of Treg development and function and indicates a genetically and developmentally programmed mechanism of Treg development.

3.3 Th17:

Recently identified as a new group of effector CD4 cells, that differentiate independently of Th1 or Th2 lineage and produce proinflammatory cytokines that lead to immune pathologies and exacerbate the autoimmune disorders, are the Th17 cells. Experiments with murine models of autoimmunity revealed an ablated disease development in mice deficient in IL23 contradicting the paradigm that these autoimmune pathologies are a result of Th1 mediated immune response. Mice deficient in IFN-γ remained susceptible to EAE and collagen induced arthrits (CIA) whereas, mice deficient in IL23p19 subunit, or mice lacking IL12p40 subunit that is shared by IL23, are protected against the
diseases. Moreover, mice lacking IL12R are also susceptible to EAE or CIA, emphasizing the involvement of a Th1 independent mechanism in disease progression (Cua et al., 2003, Murphy et al., 2003). Studies showing that IL23 induces production of IL17 from effector and memory cells and there is diminished number of IL17 producing cells rather than IFN-γ producing cells in collagen immunized IL23p19 deficient mice established the role of Th17 cells in disease development (Aggarwal et al., 2003). They also establish Th17 and Th1 as two distinct subsets developing under the effect of two different cytokines, IL23 and IL12 respectively. However, recent reports using various in vitro culture conditions have discovered that TGF-β and IL6 work synergistically to initiate the differentiation of naive T cells to Th17 cells (Bettelli et al., 2006, Mangan et al., 2006, Veldhoen et al., 2006). Nevertheless, using an in vivo bacterial infection model it has been demonstrated that though IL23 is not required for Th17 commitment, it is essential for a protective Th17 response (Mangan et al., 2006). This indicates that IL23 might be critical for Th17 mediated actions subsequent to lineage commitment. Another closely related cytokine of the same family as IL23, IL27, however potently suppresses the development of IL17 producing effector cells (Batten et al., 2006, Stumhofer et al., 2006).

4. Cytokines and Inflammatory Responses:

The activation of macrophages and other innate immune components is the first action of the host defense system upon encountering a potential pathogen. Hereby, these innate immune cells acquire microbicidal effector functions and secrete proinflammatory cytokines. The resulting inflammation is characterized
by recruitment of various immune cells to the site of infection and subsequent clearance of the pathogen by phagocytosis and/or release of microbicidal molecules. The inflammatory process is sculpted and regulated by various cytokines, of which IFN-γ, IL4 and IL10 are critical, the first two mediating different forms of inflammatory responses and IL10 has the ability to suppress both these types of inflammation.

4.1 IFNγ and Inflammation:

The indispensable role of IFN-γ in host innate defense against microbial pathogens is demonstrated using gene disrupted mice for IFN-γ, IFNγR (both 1 & 2) and STAT1. IFNγ binds to IFNγR and transduces its signal through STAT1 for proper expression of IFNγ inducible genes (Schroder et al., 2004). Mice lacking IFNγ, its receptors and STAT1 are severely impaired in their ability to clear various intracellular pathogens (Shtrichman & Samuel, 2001). Most of the effector function modulation of the innate immune cells by IFNγ comes from its ability to activate the macrophages. IFNγ stimulated macrophages show enhanced pinocytosis and receptor mediated endocytosis. Their microbicidal activity is also improved and they clear bacteria, viruses, protozoa and tumors more efficiently. The major effector molecules involved in these clearance mechanisms are the reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI). These are low molecular weight, lipophilic, reactive molecules that can easily penetrate the cell wall of the microbes and inflict injury (Schroder et al., 2004). IFNγ induces the NADPH-dependent phagocyte oxidase system, a process referred to as respiratory burst, and facilitates the
generation of ROI (Cassatella et al., 1990). RNI production is stimulated by IFNγ at different levels through the upregulation of expression of substrate, cofactor and catalyst required for NO generation (Drapier, Wietzerbin & Hibbs Jr., 1988, Kwon, Nathan & Stuehr, 1989, Di Silvio et al., 1993). The importance of these molecules in host defense is emphasized by the fact that mice doubly deficient for NADPH-oxidase and iNOS are highly susceptible to pathogens (Shiloh et al., 1999). IFN-γ also enhances the expression of FcγR1 on mononuclear phagocytes that improves the antibody dependent, cell mediated cytotoxicity (Erbe et al., 1990). Complement secretion and cell surface expression of complement receptor on the surface of mononuclear phagocytes is further enhanced by IFNγ (Strunk et al., 1985). Moreover, IFNγ activated macrophages show more rapid and greater response to LPS and other TLR ligands like CpG DNA from the bacteria. Mice with disrupted IFNγR gene are reported to be more resistant to LPS induced toxicity (Car et al., 1994). LPS and IFNγ induced signals synergize to induce better transcription of genes such as IRF (interferon regulatory factor) 1, IP (IFNγ inducible protein) 10, ICAM (intercellular adhesion molecule) 1 and iNOS that contain STAT1 and NFκB binding site (Sims et al., 1993, Caldenhoven et al., 1994, Look, Pelletier & Holtzman, 1994, Jahnke & Johnson, 1994, Ohmori & Hamilton, 1995, Ohmori, Schreiber & Hamilton, 1997, Gao et al., 1997, Pine, 1997).

Apart from this IFNγ is crucial in trafficking of lymphocytes to the site of inflammation through upregulation of expression of various chemokines and adhesion molecules. Normally, in the absence of any immune challenge, resting lymphocytes are circulating between the blood and lymph, whereas under circumstances of localized inflammation, are selectively recruited to the site of
inflammation. This recruitment is orchestrated by the local cytokine milieu. IFN\(\gamma\), in synergy with TNF and/or IL1 promotes the enrichment of these cells in the area through the upregulation of chemokines, such as IP10, MCP (macrophage chemotactic protein) 1, MIG (monokine induced by IFN\(\gamma\)), MIP (macrophage inflammatory protein) 1\(\alpha/\beta\), RANTES, and adhesion molecules like ICAM 1 and VCAM (vascular cell adhesion molecule) 1 (Boehm et al., 1997).

Upregulation of MHC I and MHC II molecule expression on the surface of APCs is another feature of IFN\(\gamma\) that improves the efficiency of host defense mechanisms. IFN-\(\gamma\) upregulates several key players of the class I antigen presentation pathway and potentiates the cytotoxic T cell recognition of foreign peptides. It stimulates a substitution of the constitutive proteasomal subunits with ‘immunoproteasome’ subunits. Treatment of cells with IFN\(\gamma\) induces expression of new subunits, LMP2, MECL-1, and LMP7 that replace the \(\beta1, \beta2\) and \(\beta5\) subunits of the proteasome that are normally expressed in resting cells (Groettrup et al., 1996, Boehm et al., 1997). The transporter associated with antigen processing (TAP) is responsible for the transport of peptides from the cytosol to the lumen of endoplasmic reticulum for loading on MHC I molecules. Both the chains of TAP (TAP1 & TAP2) are IFN\(\gamma\) inducible. Tapasin and gp96 are chaperones that act as peptide carriers and facilitate the loading of the peptide on MHC I and protect the peptide from degradation. Both these proteins are transcriptionally upregulated by IFN\(\gamma\). IFN\(\gamma\) also plays an important role in class II antigen presentation pathway and is the only IFN known to upregulate MHC II expression. Professional APCs such as dendritic cells DCs, B cells and
macrophages show enhanced expression of MHC II upon IFNγ stimulation (Boehm et al., 1997). IFNγ can also induce MHC II expression in non-professional APCs (Billiau et al., 1988). Other components of the class II antigen presentation pathway that are required for the normal expression of MHC II on the cell surface are upregulated by IFNγ. These include invariant chain (Ii), cathepsins and lysosomal proteases that are involved in peptide production as well as DM that controls the peptide accessibility to the peptide binding groove of MHC II (Kern et al., 1995, Chang & Flavell, 1995, Lah et al., 1995, Lafuse et al., 1995, Mach et al., 1996, ).

4.2 IL4 and Inflammation:

Atopic disorders like asthma, rhinitis and hayfever are characterized by increased serum levels of IgE and skin sensitization to common environmental allergens (Kawakami & Galli, 2002). IL4, a cytokine secreted by Th2 cells, is necessary for IgE isotype switching from IgM. IL4 transgenic mice show enhanced levels of IgE and develop allergic disease with ocular lesions (Tepper et al., 1990). It is responsible for the eosinophilia, hyper-IgE and mastocytosis observed during helminth infections (Urban et al., 1991). IL4 deficient mice show normal B and T cell development but there is strong reduction in IgE levels after infection with Nippostrongylus brasiliensis, a nematode (Kuhn, Rajewsky & Muller, 1991). IL4 upregulates MHC II, costimulatory molecules CD80 and CD86 on B cells and increases their antigen presenting capability (Seder & Paul, 1994). Adhesion molecule VCAM1 expression by the endothelial cells is also upregulated by IL4 (Thornhill et al., 1991). This enhances the
trapping of cells T cells, basophils, eosinophils and monocytes on the endothelium that is a characteristic feature of allergic inflammatory reaction. However, recent evidence suggests that IL4 is more important during the initial phases of the induction of the immune response and other Th2 cytokines like IL13 play a prominent role during established allergic inflammation. Studies on the allergic inflammatory response due to gut infection by a nematode demonstrate that only IL13 is required for clearance of the worm. IL4 does not seem to be critical in the removal of the parasite as anti-IL4 treated and IL4 deficient mice were as resistant to *N. brasiliensis* infection as their wild type counterparts (Madden et al., 1991, Kopf et al., 1993). However, IL4Ra deficient, STAT6 deficient, and IL4/IL13 deficient animals were more susceptible to infection than those deficient in IL4 alone (Barner et al., 1998, Urban et al., 1998, McKenzie et al., 1999). This confirmed the major role played by IL13 in worm expulsion. Nevertheless, IL4 is required in the development of the immune response to the parasite. Similarly, anti-IL4 treated and IL4 deficient mice show attenuated airway hyperresponsiveness and other features of allergic inflammation but neutralization of IL4 during established allergic inflammation had little effect (Brusselle et al., 1995, Corry et al., 1996). On the other hand, neutralization of IL13 with siIL13Rα2-Fc treatment substantially reduced airway hyperresponsiveness (Wills-Karp et al., 1998, Grunig et al., 1998).

### 4.3 IL10 and Inflammation:

The primary function of IL10 is to keep the immune responses in check and preventing the host from the detrimental effects of the inflammatory responses subsequent to pathogen encounter. This is brought about by the various anti-
inflammatory functions of IL10 affecting various cell types and suppressing the production of almost all the proinflammatory cytokines and chemokines. The first phase of the inflammatory response is governed by the activities of monocytes and macrophages and subsequently by the APCs and T cells. IL10 inhibits the secretion of proinflammatory cytokines such as TNF-α, IL6, IL12 by the monocytes and macrophages (Moore et al., 2001). The major functions of monocytes and macrophages that are regulated by IL10 can be categorized into three groups- (1) production of cytokines and chemokines that induce inflammation, (2) antigen presentation and (3) phagocytosis. IL10 inhibits the IFNγ induced release of proinflammatory cytokines like IL1, IL6, IL8, G-CSF (granulocyte-colony stimulating factor), GM-CSF (granulocyte monocyte-colony stimulating factor), and TNF-α (De Waal Malefyt et al., 1991a, Fiorentino et al., 1991). Inhibition of IL1 and TNF-α is an important anti-inflammatory function of IL10 as these cytokines synergistically amplify the inflammatory responses by inducing a number of chemokines and other secondary mediators. IL10 also inhibits the production of various chemokines like MCP 1, MCP-5, Mip 1, Mip2, Mip3, RANTES, IP10, IL8 etc. by activated monocytes (Moore et al., 2001). These chemokines are important for the recruitment of various cell types at the site of inflammation. Besides suppressing the production of these molecules, IL10 enhances the expression of their natural antagonists like IL1 receptor antagonist (IL1RA) and soluble TNFR (Jenkins et al., 1994, Joyce et al., 1994, Hart et al., 1996). IL10 neutralizes the monocytes by inhibiting the expression of IL1RI and II by activated monocytes (Jenkins et al., 1994, Dickensheets & Donnelly, 1997). Monocyte differentiation to DCs, which are the most important APCs in a primary immune response, is prevented
by IL10 (Buelens et al., 1997, Allavena et al., 1998, Banchereau & Steinman, 1998). IL10 inhibits IFNγ and IL4 induced expression of MHCII, adhesion molecule ICAM 1, CD80 and CD86 on activated monocytes, thus reducing the antigen presenting capabilities of these monocytes significantly (Moore et al., 2001). Although IL10 enhances the phagocytic activity of monocytes/macrophages by upregulating FcγR, it reduces their ability to kill the pathogens by inhibiting the generation of superoxide radicals and NO (Cenci et al., 1993, Capsoni et al., 1995, Spittler et al., 1995, Kuga et al., 1996, Roilides et al., 1998). This is a consequence of inhibition of IFNγ and TNF-α production by IL10 (Flesch et al., 1994). The effect of IL10 on granulocytes resembles its effect on monocytes and macrophages. IL10 inhibits the synthesis of proinflammatory cytokines by granulocytes and promotes the synthesis of anti-inflammatory mediators (Cassatella et al., 1993 Kasama et al., 1994). It also inhibits the production of various chemokines by granulocytes as well as the production of prostaglandin E2 by monocytes and macrophages by inhibiting the synthesis of cyclooxygenase 2 (Niiro et al., 1994, Mertz et al., 1994, Niirro et al., 1995, ). IL10 exercises its effects on DCs by inhibiting the synthesis of IL12 by activated DCs, which is a prerequisite for specific cell-mediated immune response. The expression of MHC II and co-stimulatory molecules by DCs is also inhibited by IL10, thus compromising their antigen presenting capabilities (Moore et al., 2001). IL10 treatment of DCs can induce a state of anergy in alloantigen or peptide antigen activated T cells, which cannot be reversed by addition of IL2 or stimulation with αCD3 and αCD28 (Groux et al., 1996).

In addition to the indirect effect on the cells through APCs, IL10 can affect the T cell responses directly. CD4 T cell proliferation and cytokine synthesis is
markedly inhibited by IL10. Both Th1 and Th2 cytokines (IFNγ, IL2, IL4, and IL5) are inhibited by IL10, although the inhibitory effect on Th1 cells is more apparent (Groux et al., 1996, Asadullah et al., 1998). Presence of IL10 during the activation of T cells leads to development of cells with regulatory phenotype that secretes IL10 (Groux et al., 1997, Zeller et al., 1999, Levings et al., 2001). This phenotype is transferable to other cells with the same antigen specificity, although the proposed mechanism is cell-to-cell contact dependent (Jonuleit et al., 2000).

The inhibitory effects of IL10 on production of proinflammatory cytokines and various cell types suggest its role in \textit{in vivo} infection circumstances. Its protective role in endotoxemia has been demonstrated by the fact that BALB/c mice treated with IL10 are better protected to LPS induced toxic shock, which is characterized by reduced serum TNF levels (Howard et al., 1993, Gerard et al., 1993). This is confirmed by the increased susceptibility of αIL10 monoclonal antibody treated or IL10/- mice, which succumb to twenty fold lower doses of LPS than wild type mice (Ishida et al., 1993, Berg et al., 1995). SCID mice that are deficient in T and B cells show compromised resistance to \textit{Listeria monocytogenes} upon administration of IL10 (Tripp, Beckerman, Unanue, 1995, Kelly & Bancroft, 1996). On the other hand, αIL10 treated or IL10/- mice can control \textit{Listeria} infection more rapidly (Wagner et al., 1994, Dai, Kohler, Brombacher, 1997). Similar augmentation in early immune responses was observed in infection models of \textit{Candida albicans}, \textit{Toxoplasma gondii} and \textit{Trypanosoma cruzi} (Moore et al., 2001). IL10 treatment has been found beneficial in models of EAE (Rott, Fleischer & Cash, 1994), pancreatitis (Van Laethem et al., 1995), diabetes mellitus (Zheng et al., 1997) and arthritis.
The inhibitory effect of IL10 is also crucial in allergic responses. IL10 can inhibit the survival and cytokine production by LPS activated eosinophils (Takanshi et al., 1994) as well as inflammatory cytokines like TNF and IL6 by stimulated mast cells (Arock et al., 1996, Marshall et al., 1996). These findings are confirmed by in vivo studies in which a simultaneous intra nasal dose of IL10 with antigen challenge to previously sensitized mice abrogates eosinophilia and neutrophilia as well as TNF production that are normally observed upon antigenic restimulation (Zuany-Amorim et al., 1995). The critical role of IL10 in allergic responses is underscored by the observation that asthmatic patients have considerably low levels of IL10 in the lungs (John et al., 1998). Exaggerated airway inflammation was observed in IL10-/- mice consequent to repeated Aspergillus fumigatus inhalation as compared to wild type mice. Lung cells from these IL10-/- mice produced enhanced levels of inflammatory cytokines like IL4, IL5, and IFN (Grunig et al., 1997).

5. Inflammatory Bowel Disease (IBD):

IBD is a collective term used for Crohn's disease and ulcerative colitis. Crohn's disease is mainly centered in the ileocecal area and is characterized by transmural, patchy granulomatous inflammation. Ulcerative colitis, on the other hand, is limited to the large intestine (Papadakis & Targan, 2000). Many mouse models that resemble IBD in humans have been developed to study the role of various immune mediators in the pathogenesis of the disease. These include gene disruption and transgenic models, use of haptenating agents like tri-nitro benzene sulfonic acid (TNBS) and oxazolone to induce colitis as well as other agents like dextran sulfate sodium (DSS), and induction of colitis by transferring
regulatory T cell deficient T cell population into lymphopenic hosts (Strober, Fuss & Blumberg, 2002). However, this inflammatory process is a result of reactivity of the host defense system to the normal gut microflora as IBD does not develop in mice kept under germ free condition (Bouma & Strober, 2003). The inflammatory process involves various immune mediators like T cells, cytokines, and innate immune components.

5.1 Cytokines in IBD:

Both types of T helper cells, Th1 and Th2, can mediate the T cell responses during IBD. Differential cytokine profiles may also be dependent on the causative agent of the disease in mouse models. For example, TNBS induced IBD characteristically illustrates an IL12 mediated Th1 response which is distinguished by cellular infiltration and granuloma formation. Administration of αIL12 antibodies abrogates disease development (Neurath et al., 1995). By contrast, oxazolone induced colitis shows greater infiltration of neutrophils (Boirivant et al., 1998) and is mediated by Th2 cytokines. A role of NKT cells that produce IL13 upon activation is suggested in oxazolone induced IBD and elimination of NKT cells or blockade of IL13 by an IL13Rα2-immunoglobulin fusion protein inhibits development of colitis (Heller et al., 2002). The dichotomy between Th1 and Th2 mediated immune response is also observed in human IBD. Crohn's disease is distinctively mediated by Th1 cytokines whereas a Th2 response is prevalent in ulcerative colitis. Macrophages isolated from patients with Crohn's disease produce enhanced amounts of IL12 ex vivo, whereas macrophages from patients with ulcerative colitis show lower levels of IL12 as compared to those from normal individuals (Liu et al., 1999). STAT4
and T-bet expression, required for IL12 signalling and IL12Rβ chain expression, are elevated in T cells isolated from tissues of patients with Crohn's disease, indicating a Th1 dependent mechanism of tissue inflammation (Parrello et al., 2000, Neurath et al., 2002). These T cells or T cell clones derived from such patients produce high amounts of IFNγ and markedly low levels of IL4 (Fuss et al., 1996, Parronchi et al., 1997). Treatment of Crohn's disease patients with antibody specific for IL12p40 leads to rapid decline in inflammation in most of the patients (Mannon et al., 2004). In patients with ulcerative colitis, on the other hand, there is no evidence of elevated levels of IL4 or IL12, however, IL5 production by lamina propria lymphocytes is observed (Fuss et al., 1996). In addition to this, ulcerative colitis is associated with production of various antibodies of the IgG1 and IgG4 subclasses, which is indicative of a Th2 mediated immune response (Kett, Rognum & Brandtzaeg, 1987). However, mice deficient in IL4 show slower disease progression as compared to wild type in a DSS induced colitis model. IL4/-/- mice showed less weight loss and other clinical signs appeared late (Stevceva et al., 2001). IL4 producing cells have been reported to be present at the base of lamina propria when DSS is used to induce colitis (Dieleman et al., 1998). Other proinflammatory cytokines like IL1, IL6, TNFα, IL15, IL16, IL18 etc. are expressed at increased levels in both Crohn's disease and ulcerative colitis (Bouma & Strober, 2003).

Another important cytokine implicated in prevention of IBD is IL10. The vital role of this anti-inflammatory cytokine in colitis is illustrated by spontaneous development of colitis in mice deficient in IL10 (Kuhn et al., 1993). The mutant mice suffer from chronic inflammation that involves the entire intestinal tract, the duodenum, proximal jejunum, and proximal colon. The importance of IL10
in IBD is also strengthened by the treatment of TNBS induced colitis by administration of IL10 or adenoviral vectors expressing IL10 (Duchmann et al., 1996, Lindsay et al., 2002). Adoptive transfer of naive CD45RB^hi cells, lacking regulatory T cells, into severe combined immunodeficient (SCID) mice leads to the development of colitis. Co-transfer of CD45RB^lo cells, which have regulatory T cells that produce IL10, prevents inflammation and development of disease (Powrie et al., 1993, Powrie et al., 1994). Furthermore, CD45RB^hi cells isolated from IL10 transgenic mice expressing IL10 under the IL2 promoter failed to transfer colitis, but rather inhibited colitis induced by wild type CD45RB^hi cells (Hagenbaugh et al., 1997). The importance of IL10 in DSS induced colitis has been demonstrated by oral administration of mIL10 secreting Lactococcus lactis. This treatment has been shown to effectively reduce DSS induced colitis and prevent the development of spontaneous colitis in IL10/- mice (Steidler et al., 2000). Taken together these studies underscore the essential role of IL10 and regulatory T cells in IBD.

5.2 DSS Induced Colitis: A Simple and Reproducible Model of Colitis:

To elucidate the role of different immune components in the development of IBD in humans, a number of animal models that resemble the disease have been developed. Amongst these, induction of colitis by administration of DSS in drinking water, ad libitum, had been one of the most widely used and reliable methods. The simplicity of this method and its marked resemblance with human IBD renders it the advantage of study. While the other exogenous agents that induce colitis in murine models like TNBS and oxazolone are administered
through enema or intramural injection, DSS can be simply added to the drinking water (Bouma & Strober, 2003). DSS can induce acute colitis that is similar to ulcerative colitis in humans. However, discontinuing DSS treatment or administration of DSS in cycles leads to a partial recovery from the disease and persistence of a chronic inflammatory environment that resembles Crohn's disease in humans (OKayasu et al., 1990). DSS most likely acts as a mucosal toxin as colitis develops even in immunodeficient mice, irrespective of the presence of lymphocytes and other immune cells in the region (Dieleman et al., 1994, Axelsson et al., 1996). Therefore, it facilitates the study of various immunomodulators in acute and non-acute colitis. Although a number of studies support the prevalence of Th1 responses in causing intestinal inflammation in DSS induced colitis, studies have indicated a possible role of Th2 immunity in mediating the inflammatory responses, as well. The importance of Th1 responses was demonstrated in acute phase of colitis, where colitis was characterized by upregulation of proinflammatory cytokines like TNF-α, IL1, IL12 and IFNγ. Increase in the dose of DSS led to further enhancement in the mRNA expression of these cytokines. Another notable increase was in the IL10 mRNA expression with increase in DSS concentration. However, IL4 expression was unaffected in this study of acute colitis (Egger et al., 2000). Another study in which DSS was administered to induce colitis in Peroxisome proliferator-activated receptor γ (PPARγ) heterozygous mice, reported increased susceptibility of these mice to DSS induced colitis as compared to WT mice. Here, treatment with PPARγ ligand reduced the disease and was associated with reduction in IFNγ and TNF-α levels. Increase in IL4 and IL10 was observed consequent to treatment of the disease (Saubermann et al., 2002).
In contrast to these studies, Dieleman et al. reported complete lack of IFNγ production in organ culture from colons of mice in the acute phase of colitis. However, IFNγ production was observed in the chronic phase, accompanied with IL4 production as observed by immunohistochemical staining (Dieleman et al., 1998). Similarly, mice deficient in IL4 have been reported to show slower disease progression as compared to wild type in a DSS induced colitis model (Stevceva et al., 2001). Therefore, a role for Th2 immune response in inflammation during DSS induced colitis cannot be completely ruled out.

6. Tumor and Immune Response:

The role of immune system in cancer extends from the hypothesis of cancer immunosurveillance, which proposes the essential role of immune system in protective responses against cancers. However, recent studies in the immunosurveillance against tumors suggest a three-phase immune response against tumors collectively termed as cancer immunoediting. This envisages the elimination, equilibrium, and escape as the three steps of this process that not only enable the immune system to eradicate the immunogenic tumors but also promote the emergence of tumors with lower immunogenicity and facilitate their recognition and escape form the immune system. Elimination step represents the cancer immunosurveilance by the host defense mechanism while equilibrium is the latent phase of immune response that follows incomplete destruction of tumor in the elimination phase and escape is the outcome in which tumors that have surpassed the immunological barrier grow without any restraints (Dunn, Old & Schreiber, 2004). Moreover, tumor cells employ several indigenous mechanisms that facilitate immune evasion. Tumor cells express extremely low
levels of MHC and costimulatory molecules and express weak antigens on the cell surface. In addition to this, they produce various immunosuppressive factors like IL10, TGF-β, PGE2 etc. that systemically suppress the immune effector functions (Kim, Emi & Tanabe, 2005).

6.1 Tumor Immunosurveillance and Mechanisms of Tumor Elimination:
The cellular immune components involved in protecting the host from tumors belong to both the adaptive and innate immune compartments. That the adaptive immune system plays a critical role in tumor immunosurveillance was demonstrated in a chemical carcinogen induced tumor model using recombinase activated gene 2 (RAG2) deficient mice. RAG2-/- are deficient in αβT cells, B cells, NKT cells and γδT cells in the peripheral lymphoid compartments, as they cannot somatically rearrange lymphocyte antigen receptors. Subsequent to tumor induction by subcutaneous injection of a chemical carcinogen, 3'-methylcolanthrene (MCA), RAG2-/- mice developed tumor much faster and at greater frequencies than the wild type. Spontaneous tumor development has also been reported to be more prevalent in RAG2-/- mice than in their wild type counterparts (Shankaran et al., 2001). The relative contribution of different T cell subsets has been assessed using mice that are deficient in αβ T cells (TCRβ-/-) or γδ T cells (TCRδ-/-). Mice lacking either of the TCRs are more susceptible to MCA induced tumor formation than the wild type controls suggesting an important and non-redundant role of αβ and γδ T cells in host defense against tumor (Girardi et al., 2001). However, using a 7, 12-dimethylbenzanthracene
(DMBA) and 12-O-tetradecanoylphorbol 13-acetate (TPA) to induce skin tumor, it was found that host protection is more dependent on γδ T cells than αβ T cells, as TCRδ-/- mice were more susceptible to tumor formation than the wild type, whereas TCRβ-/- were not (Girardi et al., 2003). The importance of CD8 T cells in extending protection against tumor has been determined using a murine melanoma model as well. B16, a poorly immunogenic melanoma line, was cultured in presence of IFNα for two weeks and used to vaccinate mice. These vaccinated mice showed protection against B16 tumor challenge, which was abrogated upon CD8 T cell depletion using monoclonal antibody. Specific knockout experiments in this study also revealed the importance of CD4 T cells in antitumor immunity (Wu & Fleischmann Jr., 2001).

The innate component participating in tumor immunosurveillance mainly consists of NK cells and NKT cells. Evidence in this regard comes from the experiments in which NK cells and NKT cells were depleted using a NK1.1 monoclonal antibody. These mice were two to three times more susceptible to MCA induced tumorigenesis than the wild type. Similar observation was reported following anti-asialo-GM1 treatment that depletes mice of NK cells as well as activated macrophages, indicating the importance of innate immune components in host protection against tumor (Smyth, Crowe & Godfrey, 2001). NK cell mediated protection against poorly immunogenic B16 has been demonstrated by inducing tumor using B16 cells into CD8α-/- mice. In this study immunization of WT and CD8α-/- mice with DCs engineered to express MART1 melanoma antigen led to a significant protective response against B16 tumor in WT mice. However, enhanced antitumor response was observed in CD8α deficient mice that were unable to mount any CD8 mediated protective
response (Ribas et al., 2004). This study also emphasizes the role of CD8 T cells in protection against tumor as WT immunized depleted of CD8 T cells using a monoclonal antibody show enhanced tumor progression as compared to mice in which CD8 positive population was intact. The role of NKT cells in tumor rejection was confirmed by studies with Jα281/- mice lacking a large population of Vα14Jα281 expressing NKT cells. These mice showed higher incidence of MCA induced tumor formation than wild type control mice (Smyth et al., 2000). Another notable study emphasizing the role of NKT cells in protection against tumor demonstrated reduced frequency of MCA induced tumor formation and longer latent phase in tumor development in mice treated with α-galactosylceramide (α-GalCer), a NKT cell activating ligand, as compared to control mice (Hayakawa et al., 2003). Many groups have also evaluated role of other innate immune components in imparting protection against tumor. Macrophages have been shown to be important for antitumor response and depletion of macrophages led to an inhibition in responses against tumor in a B16 melanoma model, albeit they were not involved in direct cytolytic activity (Buhtoiarov et al., 2006). However, macrophages derived cytokines and their antigen processing and presentation capabilities have been proposed to be important for an effective antitumor response. More recently, two independent groups who have identified an atypical dendritic cell population different from the conventional and plasmacytoid DCs have provided evidence regarding involvement of other innate immune cells in offering protection against tumor. These DCs co-express NK cell markers on the surface and are B220 positive, a marker of B cells. These cells are specialized to secrete high amounts of IFNγ and mediate tumor cell lysis, hence are termed as interferon
producing killer dendritic cells (IKDC) (Taieb et al., 2006, Chan et al., 2006). Using a B16.F10 melanoma model Taieb et al. demonstrated that inoculation of B220+NK1.1+ DCs into established tumor led to a significant decrease in tumor progression as compared to B220-NK1.1+ cell injection. They also propose that this IKDC population is the major IFNγ producers and not the NK cells.

6.1.1 Effector Mechanisms of Tumor Lysis:
Tumor cell lysis by effector immune cells is mediated by one of the two distinct pathways identified up till now. One of these pathways is initiated by Fas associated death domain (FADD) through receptor activation on tumor cells. The other pathway involves secretion of cytolytic molecules such as perforin and granzyme by the effector cells. The most important receptor that recruits the FADD and activates the caspase cascade is Fas. Other receptor mediated death pathways that involve TNF receptor family like TNFR1 and TRAILR2 also employ the FADD and caspase cascade leading to tumor cell apoptosis (Russel & Ley, 2002). Constitutive expression of TRAIL on a subset of NK cells in the liver has been reported, and it is known to be IFN inducible in monocytes, NK cells and dendritic cells. TRAIL deficient or WT mice treated with neutralizing antibodies to TRAIL show higher incidence of MCA induced tumor than the WT controls. In addition to this, p53 heterozygous C57BL/6 mice treated with the same neutralizing antibody to TRAIL developed more spontaneous tumors with age than the control IgG treated mice (Dunn, Old & Schreiber, 2004).
6.1.1.1 Perforin/Granzyme mediated cell lysis:

The perforin dependent tumor cell lysis is prominently exhibited by CD8 T cells and NK cells. The NK cells contain pre-formed granules and thus can kill cells rapidly upon receptor mediated activation. As the NK cells are a part of the innate immune system, they show poor proliferation upon activation. Hence, they display a quick response but have limited capacity to clear large antigenic load. On the other hand, CD8 T cells start expressing granule components consequent to TCR mediated activation. However, they proliferate upon activation and can eliminate a larger number of transformed cells. The perforin/granzyme dependent tumor cell lysis requires cell to cell contact between the effector and target cells. This contact is initiated by the activating receptor and is maintained with the help of adhesion molecules. The CTLs and NK cells then release their granule components into the intercellular space (Russell & Ley, 2002). These molecules are highly cytotoxic and the effector cells have developed useful mechanisms to prevent themselves and the neighbouring cells from the harmful effects. The secretory organelles in which these proteins are stored direct themselves to the cell surface and expose their contents only upon contact with a target cell. This process is highly regulated and the cytotoxic molecules are released in the defined space at the contact site referred to as the secretory domain (Anderson et al., 2006). Perforin also reacts non-specifically with Ca++ and lipids, therefore, is easily inactivated by the Ca++ and lipids present in the extracellular space (Russel & Ley, 2002).

CD8 T cells and NK cells use specialized lysosomes as storage organelles for the cytotoxic proteins (Trapani & Smyth, 2002). The protein that regulates the trafficking of the lysosomes, known as CHS1 (Chediak-Higashi Syndrome 1) in
humans and LYST (Lysosomal trafficking regulator) in mice is crucial for the orientation and fusion of the granules to the plasma membrane at the effector-target contact region (Russel & Ley, 2002). A defect in the gene encoding this trafficking regulator leads to Chediak-Higashi syndrome in humans and beige defect in mice. Due to non-functional CHS1/LYST, granule components keep accumulating in a huge intracellular granule and are not exocytosed upon activation. As a result, CTLs and NK cells from patients with this syndrome and beige mice exhibit diminished cytotoxic activity (Spritz, 1998, Ward et al., 2000).

Perforin released from the cytotoxic granules rapidly polymerizes to form a ring like structure in the presence of Ca++ (Young, Podack & Cohn, 1986). This ring like structure is inserted into the target cell plasma membrane and has a pore in the centre. This polyperforin stimulates the target cell for membrane repair and is taken up by reparative endocytosis (Podack, 1999). NK cells also secrete platelet activating factor (PAF) that probably synergizes with perforin in causing membrane damage. IFNγ induced PAF receptor expression has been reported in perforin sensitive cell lines (Berthou et al., 2000). The importance of perforin in tumor cell lysis has been demonstrated by using a human leukemia cell line. Perforin was unable to bind to the cell surface of these tumor cells rendering them resistant to NK cell mediated killing (Lehmann et al., 2000).

The entry of granzymes into the target cells is debatable. However, more recent studies suggest that granzyme B enters targets cells via receptor mediated endocytosis by binding to cation-independent mannose-6-phosphate receptor (CI-MPR) (Motyka et al., 2000). This granzyme B moves to an endolysosomal compartment and requires a signal from internalized perforin to leave this
compartment (Browne et al., 1999). Consequent to perforin mediated release, it activates the apoptotic pathway in the target cells by cleaving cytoplasmic and nuclear apoptotic substrates (Russell & Ley, 2002). Studies, however, demarcated a differential requirement of perforin in tumor lysis using a B16.F10 melanoma model (Prevost-Blondel et al., 2000).

6.1.1.2 Antibody dependent cellular toxicity (ADCC) and complement dependent cytotoxicity (CDC):

Recognition of tumor cells, that are not promptly recognized by the innate immune cells, is also facilitated by Fcγ receptor (FcγR) mediated antibody dependent mechanism. The FcγR expressed on the surface of granulocytes, NK cells, monocytes and macrophages recognizes the Fc fragment of immunocomplexed IgGs on the target cell surface. This mechanism of target cell recognition and elimination is referred to as antibody dependent cellular toxicity (ADCC). Most of the monoclonal antibodies that incite ADCC also activate the complement system that can result in direct lysis of target cells via complement dependent cytotoxicity (CDC) (Gelderman et al., 2004). CDC may occur through any of the two pathways of complement mediated lysis, classical pathway in which complement binds after the antibody or the alternate pathway in an antibody independent manner. Activation of the complement system leads to the formation of membrane attack complex (MAC, C5b-9) that is responsible for the lysis of target cell. Complement activation also enhances ADCC. In this case, deposition of C3b that is subsequently converted to inactivated C3b (iC3b) and in some cases, of C1q or C4b on the target cell surface increases the FcγR mediated recognition by effector cells. This method of tumor cell lysis has been
termed as complement dependent cellular cytotoxicity (CDCC) (Gorter & Meri, 1999).

The significance of ADCC in protection against tumor has been studied in vivo using nude mice and FcγR deficient mice. The monoclonal antibodies most useful to confer ADCC, CDC and CDCC have been mouse IgG2a and IgG3, and IgG1 and IgG3 in humans (Wallace, Howell & Fanger, 1994). Humanized anti-HER2/neu monoclonal antibodies raised against epithelial growth factor receptor 2 displayed significantly reduced tumoricidal activity in FcγR deficient nude mice as compared to wild type nude mice (Ravetch & Clynes, 1998). Similarly chimeric anti-CD20 treatment of FcγR deficient mice results in poor tumor regression as compared to the WT (Clynes et al., 2000).

6.2 Immune Evasion by Tumors:

Although several mechanisms for tumor eradication exist, tumors have been reported to grow in immunocompetent hosts. Shankaran et al. have demonstrated that tumor derived from immunocompetent mice and immunodeficient Rag2-/- mice grow progressively when transplanted into Rag2-/- mice. However, some of the tumors derived from the Rag2-/- mice did not develop in WT mice, while the tumors derived from the WT mice grew (Shankaran et al., 2001). Additional evidence comes from reports where MCA induced sarcomas derived from nude (Svane et al., 1996) or SCID (Engel et al., 1996) mice were frequently rejected as opposed to similar tumors derived from the immunocompetent mice, after transplantation into WT mice. Deficiency of Jα281 leads to similar results. Sarcomas derived from Jα281-/- mice are rejected after transplantation in WT
host, while they grew progressively in Jα281-/- recipients (Smyth et al., 2000). In addition to this, WT recipients reject tumors derived from pfp-/- mice, as well (Street et al., 2002). These data suggested that the host immune system itself is somehow involved in shaping the immunogenecity of tumors. For this, it was hypothesized that the immune system, though initially limits tumor growth, it is unable to eradicate many of the less immunogenic and mutated variants. The mutations in the tumor cells occur through three types of genetic instabilities: nucleotide-excision repair instability (NIN), microsatellite instability (MIN), and chromosomal instability (CIN). Of these three, CIN seems to be the predominant mechanism responsible for loss of genome integrity of the tumor and may result in loss of whole chromosome observed in cancer cells (Lengauer, Kinzler & Vogelstein, 1998). This genetic instability of tumor cells potentially results in generation of many tumor variants with reduced immunogenecity.

Other than this, tumor cells adopt mechanisms to sabotage the immune system and facilitate their escape. For example, tumor cells directly or indirectly inhibit the antigen presentation pathway. Lack of surface MHC I has been reported in various human tumor specimens (Marincola et al., 2000). Murine melanoma tumor B16 is a well established tumor model for poorly immunogenic tumor due to its low levels of MHC I expression (Chiang, Henson & Stroynowski, 2003). Studies on this model of murine melanoma have involved augmentation of its immunogenicity using different strategies (Chiang, Henson & Stroynowski, 2003, Turk et al., 2004). This loss of MHC is caused by mutations in the β2 microglobulin gene and decreased expression may result due to post transcriptional regulation. In addition to this, other components of the antigen presentation pathway like TAP1 and immunoproteasome subunits LMP2 and
LMP7 are not present in many tumors (Seliger et al., 2000, Seliger et al., 2001). Physiological relevance of these subunits was demonstrated by using LMP2-/ mice in which incidence of uterine tumor was more as compared to the WT counterparts (Hayashi & Faustman, 2002). Impairment of T cell signalling has been observed in mice with advanced tumors. Tumor infiltrating lymphocytes show a marked decrease in the expression of CD3ζ chain and the Lck and Fyn tyrosine kinases, all of which are important in the proximal signalling events of T cell activation (Mizoguchi et al., 1992, Khong & Restifo, 2002). It has been demonstrated that disruption of the proximal signalling is a result of the activity of inhibitory phosphatase Shp-1 and leads to impaired CTL mediated lysis in the effector phase of the immune response (Koneru et al., 2005). Tumors also inhibit the host immune system by producing suppressive cytokines like TGFβ and IL10 (Khong & Restifo, 2002). They also secrete soluble MIC NKG2D ligands which downregulate the NKG2D receptor on the surface of NK cells and thus evade NK cell mediated cytotoxicity (Groh et al., 2002). Moreover, some tumors have been known to secrete inhibitors of T cell responses such as galectin-1 and indoleamine 2,3-dioxygenase (Uyttenhove et al., 2003, Rubinstein et al., 2004). Tumors inhibit dendritic cell maturation through a STAT3 dependent mechanism and suppress the proinflammatory responses, thus evading immune detection (Wang et al., 2004). CD4+CD25+ regulatory T cells facilitate this immunosuppressive activity of tumors. These regulatory T cells have been shown to be responsible for failure of immunocompetent hosts to reject transplanted tumors and depletion of CD4+CD25+ T cells population by a monoclonal anti-CD25 antibody led to rejection of these tumors (Shimizu, Yamazaki & Sakaguchi, 1999, Onizuka et al., 1999). Many tumors also evade
complement mediated lysis by expressing various membrane bound complement regulatory proteins such as CD46 (membrane cofactor protein-MCP), CD55 (decay accelerating factor-DAF) and CD59 (Gorter & Meri, 1999).

6.3 Cytokines and Tumor:
A variety of cytokines are produced in the local tumor environment by the tumor cells and the infiltrating immune cells. The interaction between cytokines and their receptors forms an elaborate network that is largely responsible for overall tumor progression or induction of anti-tumor responses and tumor rejection. IL12 and interferons promote anti-tumor immunity. Tumor therapy models in which tumor bearing mice were administered exogenous IL12 revealed the anti-tumor potential of this cytokine. Treatment of mice with IL12 led to rejection of transplanted tumors. Incidence of MCA induced fibrosarcomas was reduced significantly in IL12 treated mice as compared to control mice (Noguchi et al., 1996). Additional evidence for the relevance of IL12 in anti-tumor responses was obtained when MCA was used to induce tumor in IL12p40 deficient mice. These mice developed 2-3 fold more fibrosarcomas than their WT counterparts (Smyth et al., 2000). IL12 receptor is expressed on activated T cells and NK cells. This renders IL12 as a critical regulator of the early phase of the immune response by activation of NK cells (Trinchieri, 2003). IL12 also plays a role in developing the cytotoxicity of CTLs. Cells stimulated in absence of IL12 fail to express granzyme B and thus are incapable of lysing targets (Curtsinger et al., 2005). It induces IFNγ production by T cells and NK cells, hence, can promote protective immunity against tumor through IFNγ dependent mechanisms. A study demonstrating importance of IFNγ in IL12 mediated anti-tumor immunity
shows that injecting monoclonal anti-IFNγ antibodies revokes the IL12 mediated rejection of MC38 sarcoma (Nastala et al., 1994).

IFNγ has an indispensable role in protective immunity against tumor. Endogenously produced IFNγ imparts protection against growth of transplanted tumors. Injection of neutralizing monoclonal antibodies specific for IFNγ into mice that have transplanted tumor abrogates LPS induced tumor rejection. The transplanted tumor grew more rapidly in mice treated with anti-IFNγ. Formation of Meth-A induced tumors was also faster in mice treated with IFNγ specific monoclonal antibody (Dighe et al., 1994). Mice deficient in IFNGR1, or STAT1 were more susceptible to MCA induced tumor formation than the WT mice (Kaplan et al., 1998). These mice developed more tumors at a faster rate and low doses of the carcinogen. Similar results were obtained in IFNγ deficient animals confirming the major role of IFNγ in protection against tumor (Street et al., 2001). IFNγ compliments the cell mediated tumor suppression. MCA induced tumor formation in RAG2-/- or RAG2-/- STAT1-/- (RkSk) mice were not different and both groups of mice developed tumors at lower doses. However, breast tumors observed in RkSk mice that were absent in RAG2-/- mice shows that IFNγ mediated tumor suppression does completely overlap with lymphocyte mediated suppression (Shankaran et al., 2001). In genetic tumorigenesis models, mice doubly deficient in p53 and IFNGR1 or STAT1 developed more spontaneous tumors than mice lacking only p53 (Kaplan et al., 1998). IFNγ can also promote host protective immunity against tumor by inhibiting the generation and/or activation of CD4+ CD25+ Tregs (Nishikawa et al., 2005). The same group had demonstrated that immunization of WT mice with plasmids
encoding tumor self-antigens like DnaJ-like 2 induces a potent Treg response. Growth of transplanted tumors and MCA induced tumor formation both were observed in these mice. Compared to this, mice immunized with tumor self-antigen-encoding plasmids along with IFNγ developed fewer tumors and showed slow progression of tumors. Treg response was reduced in the IFN-γ treated mice (Nishikawa et al., 2003). Role of IFNγ in imparting protection against poorly immunogenic murine melanoma B16.F10 has been evaluated in a study using a gp33 expressing B16.F10 cell line to induce tumor in a TCR transgenic mouse specific for gp33. Here, the researchers have demonstrated the crucial role for IFNγ in mediating the effector phase of tumor elimination (Prevost-Blondel, 2000).

Type 1 IFNs (IFNα & β), as well, play a role in anti-tumor immunity. In vivo neutralization of type I IFNs with polyclonal goat antisera specific for type I IFNs led to enhanced growth of tumor cell xenografts and metastasis in mice (Reid et al., 1981). Allogenic or syngenic tumor challenge also led to higher growth rate of tumor and reduced survival in mice treated with anti-IFN serum as opposed to mice treated with control serum (Gresser et al., 1983). Recently the importance of type I IFNs in protection against tumor has been demonstrated using monoclonal antibodies against type I IFN receptor (Sheehan et al., 2006). Mice treated with anti-IFNAR1 antibody failed to reject highly immunogenic MCA induced sarcomas that were otherwise rejected in WT mice treated with control antibody. MCA induced sarcomas derived from IFNAR1 deficient mice were highly immunogenic and were rejected when transplanted into naive syngenic immunocompetent mice (Dunn et al., 2005). Conversely, sarcomas derived from WT mice grew progressively upon transplantation.
Other cytokines that modulate the immune response during tumor development, specifically in suppressing the anti-tumor immunity, and facilitating the escape of tumors are TGF-β and IL10. Both these cytokines exert pleotropic consequences on the immune response and inhibit T cell activation, proliferation and differentiation (Moore et al., 2001, Li et al., 2006). Transfection of TGF-β cDNA into highly immunogenic tumors results in progressively growing tumors (Torre-Amione et al., 1990). Elevated serum levels of TGF-β have been reported in a number of malignancies (Li et al., 2006). Blockade of TGF-β mediated signalling in CD8 cells have led to generation of improved tumor specific CTL response (Gorelik & Flavell, 2001). TGF-β suppresses the expression of various cytolytic effector molecules like perforin, granzyme A & B, FasL and IFN-γ. All these molecules are responsible for effective anti-tumor response. Treatment with antibody specific for TGF-β restored the expression of these molecules in tumor specific CTLs (Thomas & Massague, 2005). CD8 T cells from individuals that had received a melanoma vaccine when cultured in the presence of TGF-β exhibited repressed effector functions (Ahmadzadeh & Rosenberg, 2005). One study demonstrated TGF-β and IL13 mediated immune suppression by NKT cells (Terabe et al., 2000). Blockade of TGF-β resulted in enhanced frequencies of tumor specific CTLs and abrogated the NKT cell mediated immunosuppression. Several therapeutic approaches have emerged that involve neutralizing TGF-β mediated immunosuppression by administration of anti-TGFβR2 monoclonal antibody or by inhibiting the ATP binding site of TGFβR1 and disrupting the TGF-β signalling (Waldmann, 2006, Li et al., 2006).
IL10 suppresses the DC functionality and protects the tumor cells by inhibiting the MHC I antigen presentation pathway. It downregulates TAP1 and TAP2 as well as MHC I on the tumor cells (Kurte et al., 2004). Significant IL10 mRNA expression has been reported in melanoma and melanoma metastases but not in healthy skin (Kruger-Krasagakes et al., 1994). Dummer et al. observed similar results and reported IL10 production by a high percentage of melanoma metastases and related cell lines (Dummer et al., 1996). There are reports of biologically active IL10 production by many other tumors like B, T, NK cell lymphomas and squamous cell carcinoma (Asadullah Sterry & Volk, 2003). However, some recent reports provide evidence for an immunostimulatory role of IL10 in anti-tumor responses. Overexpression of IL10 in the tumor microenvironment leads to a synergistic effect with other cytokines and promotes tumor rejection (Mocellin, Marincola & Young, 2005, Lopez et al., 2005). Transgenic mice that overexpress viral IL10 in the epidermis show less incidence of tumor than in WT controls (Ding et al., 2003). In contrast to this, a detailed study using a B16 melanoma model provides crucial evidence for the IL10 mediated suppression of anti-tumor immune responses. Three clones of IL10 expressing B16 cells that secreted low, medium and high quantities of IL10 were obtained by transfecting IL10 cDNA. Here it was observed that proliferation and tumor growth were greater in the IL10 transfected cells as opposed to non-transfected B16 and corresponded to the concentration of IL10 secreted. Cell surface expression of MHC I was also completely abrogated in the clone secreting highest amount of IL10. Treatment with IL10 neutralizing antibody reverted the effects of IL10 in these cells (Garcia-Hernandez et al., 2002). Even the T cells infiltrating the tumor seem to convert to a regulatory
phenotype, secrete immunosuppressive cytokine like TGF-β and IL10, and express Foxp3 (Jarnicki et al., 2006, Nair et al., 2006). In addition to this, CD4+CD25+ cells infiltrating the tumor also exert an immunosuppressive effect and promote tumor escape and growth (Zou et al., 2005). Proliferation of NK cells is also inhibited by IL10. Here blockade of IL10 signalling with anti-IL10R antibody or removal of CD14+ monocytes from the culture enhanced the proliferation of NK cells (Goodier & Londei, 2000).