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The evolutionary design of the vertebrate immune system has been shaped by constant and intimate interactions with microbial flora. Microbe-vertebrate host interactions are dynamic, with both the host and the microbe bringing their repertoire of survival mechanisms to bear. The eventual outcome can be deleterious for either the host or the microbe, as happens in acute infections, or result in an apparent steady-state coexistence for long periods of time, as happens in chronic infectious diseases. Even in such an apparent compromise, the interaction can lead to disease at times when the host immune status is modified.

In order to deal with a wide variety of potential microbial interactions, the immune system uses two lines of defense. The first line of defense consists of cells of the myeloid lineage, which acts rapidly on contact with pathogen and depends on broad based pathogen pattern recognition. This then alerts and activates the highly specialized lymphoid response, which depends on specific target-based pathogen recognition resulting in a highly specific response, with eventual preservation of pathogen recognition in the form of a long-lasting memory response. These two categories of responses are separate but highly interdependent; with the generation of the lymphoid response being dependent on instruction by the myeloid cells, and the eventual pathogen clearance being affected by the lymphoid cells making use of the highly effective effector responses of the myeloid cells. Given the combination of complexity and specificity in this mosaic of host-microbe interaction, it is not surprising that some of the genes controlling these wings of immune defense are key regulators of cellular signaling responses and have global effects on multiple cell lineages involved in the immune response, while others have pathogen specific modulatory effects.
Receptor ligand interactions, whether in myeloid lineage cells subsequent to ligation of pattern recognition receptors (PRRs), or in lymphoid cells of the B and T lineage subsequent to T cell receptor (TCR) and B cell receptor (BCR) ligation, result in activation of intracellular signaling intermediates. One of the first events subsequent to receptor ligation, conserved across both these cell lineages, is the activation of receptor associated and non-receptor associated protein tyrosine kinases (PTKs) (Schmidt et al., 2004). While the receptor associated tyrosine kinases are intrinsically associated with the cytoplasmic domains of cell surface receptors, the non-receptor tyrosine kinases undergo signal induced association, and both transduce ligand-induced intracellular signals by tyrosine phosphorylation.

1. **Bruton’s tyrosine kinase**

   The Tec family is the second largest of the cytoplasmic non-receptor associated tyrosine kinases and is composed of five mammalian members: Btk, Bmx, Itk, Tec and Txk (Smith et al., 2001). Bruton’s tyrosine kinase (BTK) is expressed only in the hematopoietic lineage, and is found in all hematopoietic cells with the exception of T lymphocytes and plasma cells (Smith et al., 1994). It has multiple signaling outcomes regulating both effector functions and development of the various cell lineages it is present in, including B cells and myeloid cells. Structurally, in addition to the Src homology (SH) domain, which it shares with other Tec kinases; BTK has a characteristic pleckstrin homology (PH) domain located at the N-terminus of the molecule. The PH domains bind to membrane phospholipids, phosphatidyl inositol-3, 4, 5-triphosphate (PIP3) in case of BTK, which serves to translocate BTK to the membrane in response to phosphatidylinositol 3-kinase (PI3K) activation and directly regulates its signaling.
function (Lindvall et al., 2005). Membrane recruitment of BTK is a crucial step towards phosphorylation and activation. Abrogation of membrane recruitment due to a mutation (R28C) in the PH domain results in X-linked immunodeficiency in mice (Mohamed et al., 1999).

1.1 BTK in B cells

Membrane ligation of BCR by various cytokine receptors such as those for IL-3, IL-4 and IL-6 (Lindvall et al., 2005), or by PRRs such as TLR2 and TLR4 (Horwood et al., 2006) leads to recruitment of BTK as a downstream signaling molecule in B cells. Activated Btk then phosphorylates phospholipase C-γ2 (PLC-γ2) (Rodriguez et al., 2001) that hydrolyses PIP₃ into inositol triphosphate (IP₃) and diacyl glycerol (DAG) resulting in calcium mobilization and activation of protein kinase C (PKC) (Miller and Berg, 2002). Further downstream, BTK signalling plays an important role in degradation of inhibitory protein of the transcription factor nuclear factor-κB (NF-κB), IκBα, resulting in transcriptional activation.

1.1.1 Role of BTK in B cell development

BTK plays a crucial signaling role in B cell development, such that a lack of its functioning in XLA (X-linked agammaglobulinemia) results in greatly reduced numbers of mature circulating B cells (Conley, 1985), severe agammaglobulinemia (Campana et al., 1990) and bone marrow B cell development arrest at the pre/pro-B cell stage (Cancro et al., 2001). By comparison, the defect manifested in the murine XID or even the BTK deficient Btk⁻/⁻ mice is more subtle, with a reduction in circulating mature B cells to half the normal numbers, normal bone marrow B cell development. This is associated with a
peripheral B cell maturation block at the immature to mature B cell transition in the spleen (Kerner et al., 1995; Khan et al., 1995), resulting in an increased frequency of immature IgM$^+$ B cells and lower frequency of mature IgD$^+$ B cells (Scher et al., 1975a; Scher et al., 1975b). Also, while serum IgM and IgG3 levels show reduction, the levels of other Ig isotypes remain unaltered (Scher et al., 1975a; Scher et al., 1975b).

However, T cell depletion in XID mice manifests with a severe B cell developmental arrest and serum immunoglobulin deficiency that closely resembles human XLA, as shown by introducing the XID defect into a FOXN1-null nude phenotype (Wortis et al., 1982) or by marrow reconstitution in surgically thymectomised irradiated adult XID mice (Sprent and Bruce, 1984), indicating a role for the presence of T cells in mediating B cell development in the absence of BTK. No mechanisms mediating this role have as yet been demonstrated or proposed.

Tec, the founding member of the Tec kinase family, is expressed in T and B cells, in myeloid cells, and in liver (Sprent and Bruce, 1984) and is activated in response to BCR (Kitanaka et al., 1998) or anti-CD19 (Kitanaka et al., 1998) ligation, and by stimulation of the cytokine receptors for IL-3, IL-6 (Takahashi-Tezuka et al., 1997), stem cell factor (Tang et al., 1994). Though analysis of Tec-deficient mice did not reveal any defect in B and T cell lineages, Tec/Btk double deficient mice show an accumulation of pro/pre-B cells (Ellmeier et al., 2000), the stage where developing B cells become dependent on BCR signaling for further differentiation into IgM$^+$ B cells, suggestive of a role for Tec in regulating B cell development in the absence of BTK. Though members of the Tec family have been demonstrated to participate in both cytokine receptors (Yang et al., 2000) as well as pre-BCR mediated signaling events (Su and Jumaa, 2003), it is
unclear whether Tec kinase is activated in response to pre-BCR formation or in response to cytokines of T cell origin in B lineage cells.

Surface CD40 on B-cells can also modulate B-cell fate. Thus, while surface immunoglobulin (sIg) cross-linking results in programmed cell death, concomitant CD40 co-stimulation suppresses the same. The ligand for CD40, CD40L, is expressed in activated T cells (Foy et al., 1996), and in bone marrow stromal cells (Abe et al., 2002). XID or Btk−/− mice with an engineered CD40 deficiency display depletion of serum Ig of all isotypes, with a substantive peripheral B cell developmental arrest at the immature to mature B cell transition (Khan et al., 1997; Oka et al., 1996). From these studies, there emerges a potential but unexplored role for cross-talk between the circulating T cells and the developing B cells in the bone marrow, possibly involving CD40-CD40L interactions and Tec as the signaling intermediates.

1.1.2 Role of Btk in B cell effector functions

BTK has been shown to be a key intermediate in signaling pathways controlling proliferation, differentiation and immunoglobulin secretion by B cells. BTK deficient murine B cells proliferate poorly on stimulation in vivo with either thymus dependent antigens (Khan et al., 1995) or Type II T-independent antigens (Alugupalli et al., 2007) resulting in a compromised IgM and IgG3 secretion in the primary antibody response, but with generation of re-stimulation competent memory B cells, which respond normally in a subsequent antigenic recall (Ridderstad et al., 1996; Ridderstad and Tarlinton, 1997). XID B cells have also been shown to be unresponsive to CD38 signaling (Santos-Argumedo et al., 1995) and show decreased proliferation and Ig secretion on CD40 and BCR co-ligation (Hostager et al., 2003). One of the explanations for the poor
proliferative responses in XID B cells is the poorer generation and maintenance of intracellular calcium response following BCR ligation (Rigley et al., 1989), alluding to the role of BTK in regulating intracellular calcium stores (Fluckiger et al., 1998).

Cellular adhesion and trans-endothelial migration, induced by chemokine gradients, form the earliest inflammatory responses undertaken by immune cells. BTK, through its PH domain, interacts with filamentous actin and small GTPases of the Rho family such as Cdc42 and Rac1 resulting in lamellipodia formation and membrane ruffling, thereby affecting cell motility (Nore et al., 2000). Consistent with this role, BTK deficient murine B cells exhibited compromised adhesion and migratory response in response to chemokines, SDF1 and CXCL13 (de Gorter et al., 2007).

1.2 BTK in myeloid cells

BTK is expressed in various myeloid lineage cells including mast cells, macrophages, granulocytes, and dendritic cells. Activation of BTK is seen subsequent to stimulation through various cytokine receptors, integrin receptors and PRRs such as TLRs.

1.2.1 Role of BTK in myeloid cell development

BTK deficiency has been shown not to result in defects in the development of mast cells (Hata et al., 1998) or dendritic cells (Sochorova et al., 2007). However, mature polymorphonuclear neutrophil granulocytes (PMNs) and monocytes have been reported to be substantially lower in the XID mouse marrow, along with significantly lower numbers of total granulocyte lineage cells in the XID (Mangla et al., 2004). Whether this reflects a developmental defect in XID granulopoiesis, or is simply the result of altered
granulopoiesis because of changes in the lifespan of peripheral BTK-deficient PMNs is not yet clear.

1.2.2 Role of BTK in myeloid cell effector functions

Mast cells are crucial mediators of allergic reactions and act by secreting cytokines and inflammatory mediators such as histamines and leukotrienes, contained in preformed cytosolic granules, released by degranulation, subsequent to cross-linking of high affinity IgE receptors (FceRI) (Schmidt et al., 2004). XID and Btk<sup>-/-</sup> mice display poor anaphylactic reactions, degranulation, histamine release, and show a severe compromise in cytokine production and release of IL-2, IL-6, GM-CSF and TNFα (Hata et al., 1998). Compared to the rapid release of mediators, the late phase reactions requiring de novo synthesis of secreted mediators are more severely impaired in BTK-deficient mast cells as is IP₃ generation and Ca²⁺ release, subsequent to receptor ligation (Hata et al., 1998; Kawakami et al., 2000). These data suggest that the major target of BTK signaling during mast cell activation is likely to be induction of transcription.

Dendritic cells from XLA patients show impaired IL-6 and TNF-α production following stimulation with TLR8 agonist ssRNA, but not in response to stimulation of TLR1/2, 2/6, 3, 4 and 5 (Sochorova et al., 2007). Lipopolysaccharide (LPS) induced generation of effector molecules, reactive oxygen intermediates (ROI) (Mangla et al., 2004) and reactive nitrogen intermediates (RNI) (Mukhopadhyay et al., 1999), as well as pro-inflammatory cytokines, TNF-α and IL-1β, is compromised in XID macrophages. Compromised induction of inducible nitric oxide synthase (iNOS), the enzyme responsible for RNI generation, results in the poorer RNI generation (Mukhopadhyay et al., 1999). Further, induction of transcription factors of the NF-κB family is also
defective, with poor induction of p-50 and lack of induction of p-65 and c-Rel members, seen on LPS stimulation (Mukhopadhyay et al., 2002). These defects result in poorer microbial clearance, microfilaria (Mukhopadhyay et al., 1999) or *Escherichia coli* (Mukhopadhyay et al., 1999), *in vitro*, and decreased susceptibility to inflammatory conditions *in vivo*, such as experimental autoimmune encephalomyelitis (EAE) or dextran sulfate sodium (DSS)-induced colitis (Mangla et al., 2004).

Consistent with these data, macrophages from XLA patients also show compromised TNF-α induction on LPS stimulation and TLR2 ligation (Horwood et al., 2003; Horwood et al., 2006). XLA monocytes display poorer chemotaxis and phagocytic functions (Amoras et al., 2003), as well as nuclear translocation of NF-κB (Jefferies et al., 2003).

PMNs from XID mice show compromised RNI and ROI induction, poorer bacterial clearance *in vitro*, and poorer chemotaxis and recruitment in the absence of any defect in phagocytosis (Mangla et al., 2004). Inhibition of BTK function in human PMNs has been reported to lead to reduced neutrophil adhesion, migration and ROI generation (Gilbert, 2003 #93). XID macrophages and PMNs show an enhanced tendency to undergo apoptotic cell death in response to inflammatory signals such as TLR ligands and pro-inflammatory cytokines (Mangla et al., 2004).

2. **Host-Pathogen interactions**

The interplay between a potential host and the pathogen begins on first contact with the pathogen, and is continued through the activation of the process of immune inflammation.
2.1 *Initiation of inflammation*

Recognition of specific structural motifs of the invading pathogens, pathogen associated molecular pattern (PAMPs), by phylogenetically conserved cell surface pattern recognition receptors (PRRs), present on macrophages and dendritic cells, forms the first point of contact with pathogens (Janeway and Medzhitov, 2002). Central to the organization of an inflammatory response subsequent to pathogen recognition is the recruitment of immune effector cells, achieved by the secretion of signaling molecules known as chemokines (chemoattractant cytokines) (Premack and Schall, 1996). Chemokines are heparin binding small protein molecules of 7-10 kDa, divided into three distinct families based on the position of the amino-terminal cysteine residues that are present in all of these molecules (Strieter et al., 1996). Of these, CXC family members are specific mediators of neutrophil migration, CC family members mediate monocyte, eosinophil and lymphocyte migration, while the C family primarily recruit lymphocytes (Schluger and Rom, 1997). Additionally, specific signaling initiated on pathogen contact differentially controls the expression of one or more of the families of chemokines (Schluger and Rom, 1997). Acute bacterial infections are characterized by the preferential induction of CXC chemokines such as IL-8, resulting in a predominantly neutrophilic infiltrate (Mizgerd et al., 1996). *Mycobacteria*, which are macrophage resident intracellular pathogens, expectedly induce secretion of CC family members, monocyte chemotactic protein (MCP) and macrophage inflammatory protein (MIP), to induce macrophage infiltration (Friedland et al., 1993). Further, induction of T cell recruitment, proliferation (Taub et al., 1996) and Th-1 (by MCP) and Th-2 (by MIP-1α) differentiation (Karpus et al., 1997), has also been reported. Primed helper-T cells then act to amplify the immune response by secreting inflammatory cytokines.
2.2 Eosinophils

Eosinophils, a specific lineage of granulocytes, so named because of their eosinophil granule content, are multifunctional leukocytes recruited in response to CC chemokines (RANTES and eotaxin), as well as Th2 cytokines such as IL-4, IL-5, and IL-13, and modulate protection against parasitic helminth infections and mediate allergic diseases (Rothenberg and Hogan, 2006).

2.2.1 Eosinophil development

Eosinophils develop in the bone marrow from a common precursor they share with basophils (Boyce et al., 1995), under the influence of three classes of transcription factors; GATA-1 (a zinc finger transcription factor), PU.1 (an ETS family member) and C/EBP members (CCAAT/enhancer-binding protein family), of which GATA-1 is the most important for eosinophil lineage specification (McNagny and Graf, 2002; Nerlov and Graf, 1998; Nerlov et al., 1998). Eosinophils possess a high affinity palindromic GATA site, which is present in the GATA-1 promoter region and also in the regulatory regions of eosinophil specific genes such as eotaxin receptor, chemokine receptor-3 (CCR3) and IL-5 receptor alpha (IL-5α). Activation of this palindromic GATA site results in the eosinophil specific expression of these genes (Du et al., 2002; Yu et al., 2002; Zimmermann et al., 2000). Three cytokines, IL-3, IL-5 and GM-CSF, share a common receptor beta chain, and regulate eosinophil development by providing proliferation and differentiation signals and are under transcriptional control of GATA-1. Of these, IL-5 is the most proficient at inducing eosinophil differentiation and release form the marrow (Collins et al., 1995; Sanderson, 1992), as well as their exit from
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peripheral circulation, consequently, over-expression of IL-5 in mice results in profound eosinophilia (Tominaga et al., 1991).

2.2.2 Eosinophil functions

Eosinophils have been identified to serve as major effector cells by releasing cytotoxic granules containing cationic proteins such as major basic protein (MBP), eosinophilic cationic protein (ECP), and eosinophil peroxidase (EPO), and lipid mediators such as leukotrienes and platelet activating factor, resulting in tissue damage and heightened inflammatory responses (Rothenberg and Hogan, 2006). Receptor activation of eosinophils leads to targeted release of specific granule vesicles (Dvorak et al., 1991), which are relocated to the plasma membrane by the formation of docking complexes composed of N-ethylmaleimide-sensitive factor attachment protein (SNAP) receptors (SNAREs) located on the vesicle (v-SNAREs) and the target membrane (t-SNAREs) (Logan et al., 2002). Release of chemokine RANTES, but not cationic proteins, on IFN-γ stimulation of eosinophils, is an example of such a specific granule release (Lacy et al., 1999). ECP and MBP, besides exerting non-specific toxicity on a variety of tissues, have specific regulatory effects such as inhibition of immunoglobulin synthesis and T cell proliferative responses, and induction of mast cell degranulation, in case of ECP (Venge et al., 1999), and induction of basophil and mast cell degranulation, in case of MBP (Piliponsky et al., 2001; Zheutlin et al., 1984). EPO catalyzes halides (Cl−, Br−, I−) and nitric oxide to reactive oxygen and reactive nitrogen metabolites (NO2), which lead to oxidative stress and induce apoptosis and tissue necrosis (MacPherson et al., 2001; Wu et al., 1999). Eosinophils, under normal conditions are mostly resident in the intestinal lamina propria where they survive for up to 2 weeks (Mishra et al., 1999), and are
recruited to sites of inflammation under the influence of eosinophil specific chemokines of the eotaxin family and Th2 cytokines.

2.2.3 **Immunomodulatory effects of eosinophils**

MBP induces IgE independent mast cell degranulation, eicosanoid production and release of cytokines such as IL-18, TNF-α and GM-CSF (Piliponsky et al., 2002). Eosinophils also produce nerve growth factor (NGF), an important cytokine promoting mast cell survival and activation (Bullock and Johnson, 1996). Eosinophils have also been reported to process and present microbial and parasitic antigens, and modify Th1/Th2 commitment of the ensuing CD4 response by secreting a range of polarizing cytokines, notably IL-4 and other Th2 cytokines such as IL-5 and IL-13 (MacKenzie et al., 2001). Recent reports have indicated the ability of eosinophils to preferentially activate effector T cell proliferation (van Rijt et al., 2003), which, when examined in context of the ability of primed eosinophils to track to T cell rich paracortical zones of lymph nodes (Shi et al., 2000), implies a role for these cells in mediating an amplification of an ongoing effector T cell response. The three types of eotaxins reported, eotaxin-1, 2 & 3, all signal via the same eosinophil specific chemokine receptor, CCR3 (Daugherty et al., 1996), and have been reported to be sequentially induced on inflammation in epithelial cells, fibroblasts as well as infiltrating macrophages and eosinophils (Rothenberg and Hogan, 2006). Of these, the earliest acting eotaxin-1 & 2, in conjunction with IL-5 which activates a STAT-6 (signal transducer and activator of transcription-6)-dependent signaling pathway, together resulting in induction of tissue eosinophilia and Th2 skewed immune responses (Zimmermann et al., 2003).
2.2.4 Comparative functions of eosinophil and neutrophils

Eosinophils and neutrophils constitute the earliest responding cells recruited to sites of pathogen entry, and mediate rapid pathogen elimination utilizing pre-formed granule proteins stored in rapidly mobilizable vesicular organelles. In addition, neutrophils recognize microbial PAMPs (Hayashi et al., 2003) and initiate vigorous phagocytosis, whereas eosinophils depend on a less efficient antibody-dependent phagocytosis (Sanderson and de Souza, 1979) and largely mediate pathogen killing by granule exocytosis. Specialized granule structures of the neutrophils have well demarcated functions in effecting microbial killing. The azurophilic and specific granules mediate killing intracellularly by fusing with the phagosome, while the specific granules are also released by exocytosis and mediate microbial elimination in the extracellular milieu (Theilgaard-Monch et al., 2006). Neutrophils have also been demonstrated to generate extracellular fibres, called neutrophils extracellular traps (NETs), which physically trap bacteria on which the NET resident granule proteins and cathepsins then act to secure microbial killing (Brinkmann et al., 2004). The NETs have thus been seen to represent an efficient method to restrict bacterial spread at the site of infection and form a efficient mechanism to mediate focused lytic activity. In addition to the immediate killing and degradation of pathogens, activated eosinophils and neutrophils synthesize a host of chemokines and cytokines, which act to regulate the recruitment and function of other effector cells such as macrophages, T cells and other granulocytes including themselves (Theilgaard-Monch et al., 2004; Zimmermann et al., 2003). Whereas, eosinophils mediate the induction of a Th2 skewed response (MacKenzie et al., 2001), neutrophils secrete Th1 cytokines such as IL-12, IFN-γ, and chemokines active on Th1 cells such as...
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MIG (monokine induced by interferon-γ) and MIP-1α, resulting in a distinct IFN-γ biased Th1 response (Bliss et al., 2000; Chen et al., 2001).

2.3 Phagocytosis

Phagocytosis is the phylogenetically conserved process of ingestion of particulate ligands including microbes and cell debris > 1μm in size, mainly mediated by phagocytic leukocytes. Phagocytosis result in routing of ingested pathogen to the lysosomes where microbial death is mediated by hydrolytic enzymes and free radicals, and directs the microbial antigens to compartments containing MHC class II (MHCII) for processing and presentation to lymphocytes. Thus, phagocytosis serves a dual role: an immediate immune effector function as well as a bridge between the innate myeloid and acquired lymphoid responses.

2.3.1 Receptor recognition and signaling events

Pathogen recognition by myeloid cells is mediated by PRRs such as mannose receptors, NOD-like receptors (NLR) or Toll-like receptors, of which TLRs have been the most extensively characterized. They are expressed on the cell surface (TLRs 1 to 6 and TLR11) or intracellularly on endosomal membranes (TLR3, 7, 8 and 9), and binding of respective ligand rapidly transduces signals initiating NF-κB activation or cytokine secretion, especially type-I interferons (Akira, 2006). Cell surface resident TLRs specialize in ligands on microbial cell wall molecular motifs enabling extracellular bacterial recognition, while endosomal TLRs sample a variety of viral and bacterial nucleic acids effecting recognition of intracellular pathogens. Accordingly, TLR2, along with TLR1 & 6 recognize cell wall peptides of gram-positive bacteria and mycoplasma,
whereas TLR4 recognizes LPS present on gram-negative bacteria. TLR3 recognizes viral dsRNA, TLR7 & 8 recognize viral ssRNA, and TLR9 is the receptor for bacterial and viral CpG DNA repeat motifs and non-nucleic acids such as hemozoin (Akira, 2006). Recognition of PAMPs by the cognate TLR ligand results in receptor association of TIR (Toll/interleukin-1 receptor) domain containing adapters, MyD88, TIRAP (Toll-interleukin-1 receptor domain-containing adaptor protein), TRAM (toll-like receptor adaptor molecule-1) and TRIF (TIR domain-containing adapter inducing IFN-beta), and depending on the TLR that has been ligated, various combinations of these adaptors are recruited to mediate distinctive responses (Akira and Takeda, 2004). All the families of TLRs (except TLR3) utilize a MyD88 dependent pathway, where MyD88 activation by phosphorylation results in recruitment of members of IRAK (IL-1 receptor-associated kinase) family, which through subsequent phosphorylation events activate the NF-κB transcription factors resulting in expression of inflammatory cytokines TNF-α (tumor necrosis factor-α), IL-6 and IL-1β (Akira and Takeda, 2004; Kawai et al., 1999). In addition, TLR engagement, in a MyD88 independent pathway induce activation of the members of the transcription factor family interferon regulatory factor (IRF), mainly IRF-3 resulting in induction of IFN-β and IFN inducible genes (Kawai et al., 2001; Yamamoto et al., 2003). TLR4, 5 and 9 ligand engagement results in induction of Th1 associated cytokines such as IL-12, similarly TLR2 ligands induce Th2 responses characterized by IL-10 secretion (Dillon et al., 2004; Redecke et al., 2004). Thus, signaling events subsequent to pathogen recognition, not only induce specific effector myeloid responses, but also modulate the course of the lymphoid response.
2.3.2 *Phagosome formation and maturation*

Actual physical pathogen engulfment involves signaling machinery independent of pathogen recognition by PAMPs and include Fc receptors (Bharadwaj et al., 2001), receptors for components of the complement pathway (Zaffran et al., 1998) and scavenger receptors (Thomas et al., 2000), and mediates uptake of both microbial pathogens and apoptotic cell debris. Signaling through these receptors results in the activation of PI3K (Phosphoinositide-3 kinase) and phospholipase C, which generates IP₃ (inositol triphosphate) and DAG (diacyl glycerol) by hydrolysis of PIP₂ (phosphatidylinositol-4, 5-bisphosphate), which subsequently initiate calcium recruitment and activation of PKC mediated signaling pathways. PIP₂ plays a crucial role in mediating the massive cytoskeletal remodeling needed for pathogen uptake, by associating with members of the Rho family of GTPases, including Rho (Hackam et al., 1997), Rac1 and Cdc42 (Castellano et al., 2000; Cox et al., 1997), which mediate focal F-actin assembly at the periphagosomal region to initiate phagocytosis. ARF6, a member of the ARF family of GTPases, augments Rac functions and serves as a co-factor in inducing PIP₂ generation at the plasma membrane (Honda et al., 1999; Zhang et al., 1999). Together they induce actin filament based pseudopodia extension of the plasma membrane around the pathogen, resulting in engulfment and membrane closure mediated by several unconventional myosins (Titus, 1999), resulting in the internalization of the phagosome. These newly formed phagosomes undergo sequential fusion with endosomes and ultimately with lysosomes to effect degradation and killing of the microbe, a process termed phagosome maturation (Vieira et al., 2002). During the process of maturation, the phagosome recruits various signaling and effector molecules, undergoing increasing
acidification, culminating in the low pH lysosomes, pH sensitive acid hydrolases eventually act to ensure microbial degradation.

Though phagocytosis of microbes and apoptotic cells is mediated by the same phagocytic machinery, qualitatively different cellular responses ensue, due to specific pathogen recognition by TLRs. More importantly, microbial uptake is not mediated by TLRs, but rather by these phagocytic receptors which by themselves do not induce inflammation (Taylor et al., 2005), but can be transcriptionally induced on TLR activation (Doyle et al., 2004). Further, TLRs can also be recruited to phagosomes, and in a MyD88 and p38 MAPK (mitogen-associated protein kinase) dependent fashion, induce increased microbial uptake and phagosomal trafficking kinetics resulting in enhanced clearance (Blander and Medzhitov, 2004; West et al., 2004). Though the exact molecular mechanisms for this modulation have to be defined, they have been speculated to involve regulation of V-ATPases (vacuolar-ATPase) which mediate phagosome acidification, association of Rab GTPases which mediate vesicular trafficking, and increasing phagosome movement on the microtubule assembly resulting in enhanced phagosomal maturation (Blander and Medzhitov, 2006).

2.3.3 Microbicidal functions of phagosomes

Subsequent to pathogen recognition and internalization, pre-formed flavocytochrome b558, the core component of the NADPH oxidase system is recruited to the phagosome membrane where it forms a channel to direct electrons generated from cytosolic NADPH oxidation onto oxygen contained in the phagosome vacuole, thereby generating superoxide radicals (O$_2^-$) (Segal, 2005). Reactive oxygen- species/intermediates (ROS/ROI) are generated as reaction products from superoxides and include hydroxyl,
hydrogen peroxide, ozone, halides and superoxide itself, which are further converted into hypochlorous acid by superoxide dismutase (Chapman et al., 2002). These ROS species play an important role in pathogen clearance and the dysfunction of the NADPH oxidase system causes CGD (chronic granulomatous disease), characterized by profound susceptibility to bacterial and fungal infections and failure to clear anaerobic infections. Reactive nitrogen intermediates (RNI), including nitric oxide (NO) and its reaction products generated by constitutive and inducible expression of NO synthase enzymes also mediate important microbicidal effector functions (James, 1995). Accumulated NO gets converted to highly reactive peroxynitrite (ONOO⁻) radicals by phagosomal superoxide and affects microbicidal functions. Inducible nitric oxide synthase (iNOS) levels are also transcriptionally enhanced by TLR ligation or Type I interferon, prominently IFN-γ, resulting in conversion of L-arginine to L-citrulline and NO. RNIs produced in this fashion are important effector molecules in immune response against bacterial, fungal, helminth and protozoan infections (James, 1995).

3. **Endo-lysosomal trafficking**

3.1 **Rab and small GTPases**

Phagosomal maturation proceeds with sequential fusion of endosomal vesicles, resulting in loss of cell surface markers associated with the engulfed portion of the plasma membrane which now forms the phagosome bilipid membrane, and acquisition of markers of early endosomes, late endosomes and lysosomes (Desjardins et al., 1994; Pitt et al., 1992). Phagosome maturation involves multiple transient fusion events with endosomal vesicles, referred to as 'kiss and run' fusion, allowing for acquisition of
proteolytic and microbicidal substances by the phagosome, but preventing complete fusion with the endosomes (Desjardins, 1995; Desjardins et al., 1997). Such phagosomal fusion events with early endosomes are regulated by Rab5, Rab4 and Rab11 and late endosomal fusion is regulated by Rab7 and Rab9. Inactive GDP form of Rab proteins associate with the vesicle and dissociate by rapid phosphorylation to the GTP-bound form, to ensure the transient nature of the fusion. Prolonged fusion, induced by the GTP-state arrest of Rab5 results in formation of giant endosomes, and has been reported to interfere with impaired clearance of intracellular parasite *Leishmania donovani* (Duclos et al., 2000). Proton pump V-ATPase and lipid raft-associated domains (Dermine et al., 2001) associate with the phagosome during its interaction with early endosomes. Also, lipid raft domains play a facilitatory role in actin accumulation and fusion with late endosomes (Kjeken et al., 2004) as well as in recruitment and assembly of cytosolic domains of the NADPH oxidase complex on the phagosomal membrane (Shao et al., 2003; Vilhardt and van Deurs, 2004).

### 3.2 *Lysosomal transporter - LYST*

LYST (lysosomal trafficking regulator), belongs to a family of proteins characterized by the presence of a BEACH (Beige and Chediak-Higashi syndrome) and WD-domains in their C-terminal region and two members, LYST and Alfy, have been reported in *Dictyostelium* to be associated at different stages of endosomal trafficking (Cornillon et al., 2002). LYST has been shown to be associated with microtubules (Faigle et al., 1998) and shows a cytosolic distribution (Perou et al., 1997). However, despite the availability of the complete sequence of the *Lyst* gene, the precise function of LYST has not been characterized. Most studies have therefore focused on analysis of functional
defects due to mutations in the \textit{Lyst} gene, resulting in an immunodeficiency syndrome Chediak-Higashi syndrome (CHS) in humans and the mouse model for CHS, the \textit{Beige} (bg/bg) (Barbosa et al., 1996). CHS presents as an autosomal recessive disorder, characterized by partial albinism, recurrent bacterial infections and the accumulation of giant intracellular granules, which include lysosomes, melanosomes, cytolytic granules and platelet dense granules. The \textit{Lyst} mutation results in enlarged lysosomal compartments and reduced cytotoxicity due to defective granule exocytosis, evident in cytotoxic T lymphocytes (Baetz et al., 1995), NK cells (Haliotis et al., 1980; Roder et al., 1980) and granulocytes (Gallin et al., 1974). Early endosomes and trans-golgi network (TGN) are the main sources of membrane protein trafficked to the late endosomes. In CHS, molecules normally routed to the lysosomes from these two sources, via the late endosomes, are found to accumulate in early endosomes. This indicates a block in the vesicular transport between early and multivesicular late endosomes (Faigle et al., 1998). Multivesicular late endosomes, formed by fission of large portions of early endosomes mature by extensive material exchange with TGN and eventually fuse with pre-existing multilaminar lysosomes to form mixed compartments. These compartments undergo sorting and lysosomes are retrieved back. It is the missorting of endosomal proteins during the early to late endosomal transit that is likely to result in the consequent perturbation of lysosomal identity and functions (Faigle et al., 1998). Such a missorting could explain the relative lack of lysosomal membrane markers and the abundance of endoplasmic reticulum proteins in the CHS lysosomes. This could also account for the low levels of cathepsins, which are routed in membrane bound form from early endosomes, and are active at the low pH found in lysosomes (Nishi and Forgac, 2002). Cathepsin L (in mice) (Nakagawa et al., 1998) and cathepsin S (in humans) (Bania et al.,
2003) are necessary for proteolysis of the invariant chain of MHC II to CLIP. Therefore defective cathepsin transport due to missorting in CHS results in impaired peptide loading, and in conjunction defective transport of peptide-MHC II complexes to the cell surface, leads to defective MHC II mediated antigen presentation (Faigle et al., 1998; Huynh et al., 2004). Cathepsin G also has important microbicidal functions and its impaired function, especially in neutrophils, might account for the defective bacterial clearance observed in CHS (Gallin et al., 1974; Ganz et al., 1988).

Defects in phago-lysosomal function of the kind exhibit in the CHS and bg/bg has major consequences on effective pathogen clearance, especially in infections with intracellular pathogens, as evidenced by increased susceptibility of bg/bg mice to *Leishmania donovani* infection (Kirkpatrick and Farrell, 1982).

### 4. *Leishmania major*

Digenetic intracellular protozoan parasites of the genus *Leishmania* cause a chronic parasitic disease with a wide range of clinical presentations: cutaneous, mucocutaneous and visceral, with the involvement of multiple host and pathogen factors, genetic and environmental, in determining clinical disease outcome (Lipoldova and Demant, 2006). Extracellular infective stage, flagellated promastigotes of the *Leishmania* parasite are transmitted to human hosts during a blood-meal by infected phlebotome sandflies, which act as the invertebrate hosts. The parasites then gain access and are taken up by the host phagocytes, neutrophils, macrophages, monocytes and dendritic cells, and convert into the phagocyte resident aflagellated amastigote form. Amastigotes reside and multiply in host cell lysosomes, eventually rupturing the cell to be released and phagocytosed by uninfected cells. Since macrophages are both circulatory and tissue
resident in nature, infected macrophages can gain access to lymphoid organs, spleen and lymph nodes to set up a visceral infection, or take up residence in the dermis or mucosal tissue resulting in a localized cutaneous or the diffuse mucocutaneous form of leishmaniasis. Since the host macrophages are the major immune effector cells mediating pathogen elimination, the *Leishmania* parasite has evolved a variety of mechanisms to subvert macrophage function. Consequently modulation of macrophage function by acquired immune responses mediated largely by the CD4 subset of T lymphocytes plays a major role in affecting parasite clearance.

4.1 Pathogen uptake and residence in phagocytes

*Leishmania* parasites lack the ability to actively invade host cells and depend on phagocytic uptake by host macrophages involving complement receptors (CR1 and CR3) and pattern recognition receptors such as mannose/fucose receptors (Alexander and Russell, 1992). Phagocytosed parasites are then rapidly trafficked to and fuse with lysosomes to form the phagolysosomal residence site of the parasite, referred to as the parasitophorous vacuole (Courret et al., 2002). Phagocytosed promastigotes are highly susceptible to the action of acid hydrolases, hence rapid conversion to the hydrolase-resistant amastigote is critical for parasite survival (Dermine et al., 2000; Desjardins and Descoteaux, 1997). Therefore, rapid transport of the phagocytosed parasites to the lysosomal compartments is necessary to ensure elimination. Macrophage effector molecules, NO and ROI are also essential for parasite killing. Mice deficient for the enzyme iNOS are unable to control a *Leishmania* infection and macrophages derived from these mice are incapable of affecting a clearance of parasites in culture (Wei et al., 1995). Mice deficient in ROI generation however clear the infection after an initial period
of increased susceptibility (Murray and Nathan, 1999), indicating that ROIs play a somewhat less significant role in parasite clearance. Induction of IL-12, a dominant inducer of IFN-\(\gamma\) secretion by Th1 cells, results in enhanced clearance due to the ability of the secreted IFN-\(\gamma\) to induce iNOS and hence NO production in the macrophages, consequently, DCs derived from IL-12 deficient mice show compromised *Leishmania* clearance ability (Berberich et al., 2003).

*Leishmania* parasites have evolved multiple mechanisms to evade the early host effector responses. *Leishmania* evade the lytic effects of the membrane attack complex (MAC) of the complement system composed of complement factors C5b-C9, by the surface expression of glycosylated lipophosphoglycan (LPG) which prevents attachment of MAC on surface of infective metacyclic promastigotes (McConville et al., 1992). Surface expression of proteinase gp63 further enhances this protection by mediating cleavage of C3b component into an inactive form iC3b (Brittingham et al., 1995), which opsonises the parasite for CR3 mediated uptake by macrophages. In addition to ensuring the targeting of parasite to its cellular residence of choice, ligation of CR3 is of added advantage to the parasite as no respiratory burst activity is induced on CR3 activation (Mosser and Edelson, 1987). Since the promastigotes are vulnerable to lysosomal hydrolases, they retard endosomal maturation and endo-lysosomal fusion by an LPG mediated mechanism involving inhibition of protein kinase C (PKC) activity (Desjardins and Descoteaux, 1997). PKC also plays a key role in the induction of ROIs, consequently respiratory burst activity is also inhibited (Olivier et al., 1992). Consistent with these roles of LPG, *Leishmania* deficient in LPG expression showed poorer survival within macrophages after infection (Spath et al., 2000). *Leishmania* have also been reported to prevent the induction of cytokines involved in anti-inflammatory...
responses (IL-1β and TNF-α) (Hatzigeorgiou et al., 1996) and T cell activation (IL-12) (Piedrafita et al., 1999; Weinheber et al., 1998). Further, *Leishmania* induce overproduction of both IL-10 and TGF-β from infected murine macrophages resulting in uncontrolled parasite replication and non healing lesions (Barral et al., 1993; Kane and Mosser, 2001).

4.2 *Antigen presentation and induction of Leishmania-specific T cell responses*

Processing and presentation of parasite peptides on MHCII to CD4 T cells is essential for controlling a primary infection with *Leishmania* (Locksley et al., 1993). While presentation on MHC1 to CD8 cells does not seem to be essential in controlling a primary infection in β2-microglobulin deficient mice (Wang et al., 1993), other studies have found CD8 T cell functions to be important in controlling infection in a low-dose parasite challenge in mice (Belkaid et al., 2002) and in induction of optimal IFN-γ responses in primed CD4 T cells (Herath et al., 2003).

*Leishmania* infection has been reported to impair association of MHCII molecules with the parasitophorous vacuole, resulting in poor antigen presentation to CD4 T cells (Antoine et al., 1999). In addition, down-regulation CD80, an important co-stimulatory molecule involved in MHCII-TCR interaction, has also been observed in *Leishmania* infected macrophages (Saha et al., 1995). Ligation of surface CD40 on infected macrophages leads to activation of p38 MAPK signaling, resulting in increased activation of iNOS and improved parasite clearance (Awasthi et al., 2003); while impaired CD40-40L co-stimulation is observed in leishmania infected macrophages (Kamanaka et al., 1996; Soong et al., 1996).
Many human diseases, including infectious diseases have now been understood to develop predominantly in genetically predisposed individuals, with multiple genes of low-penetrance determining the disease susceptibility (Pharoah et al., 2002). However, since mapping of genes with such low-penetrance in humans would involve studies requiring the participation of a large number of subjects, in addition to correction for environmental and lifestyle factors, the more feasible approach of genetic analyses on disease-equivalent animal models has been utilized (Bedell et al., 1997a; Bedell et al., 1997b). These models have been useful in leishmaniasis in which robust immunological and genetic components are involved in determining disease outcomes.

4.3 *T cell response quality and Leishmania clearance*

Infection of human hosts commonly leads to the development of localized cutaneous lesions, which resolve spontaneously with the generation of a sustained lifelong immunity to re-infection. This phenotype is also shared by most laboratory mouse models as well as the natural rodent reservoirs of infection. However, certain strains, notably the BALB/c, develop systemic disease with progressive lesions, and are thought to mimic more systemic forms of the human disease such as visceral leishmaniasis (kala-azar) or diffuse mucocutaneous leishmaniasis (Sacks and Noben-Trauth, 2002). The genetic predisposition for resistance or susceptibility in mouse models of infection has been correlated with the dominance of an IL-12/IFN-γ driven Th1 response resulting in cure (in most inbred strains) and an IL-4 dominant Th2 response causing disease (BALB/c), respectively. While this paradigm has made a major contribution to understanding the principles of control of infectious agents, it also has major limitations in the context of understanding the genetic basis of human leishmaniasis. Firstly, the Th2
biased responses in the BALB/c mice do not seem to be disease specific, but are induced globally in every infection or immunization scenario (Hsieh et al., 1995). Secondly, the C57BL/6 mice clear a Leishmania major infection rapidly and the BALB/c mice succumb to a rapidly fatal leishmanial infection (Howard et al., 1980). Both scenarios are very unlike the human clinical leishmanial outcome, which results in most instances in chronic, persistent but indolent lesions, with prospects of future reactivation in disease. Thus, while this model contributes to elucidating the genetic regulation of the Th1/Th2 balance, additional putative loci that might impart qualitative regulation on the disease outcome in humans, and the genetic targets of such regulators are still poorly understood (Lipoldova and Demant, 2006). In light of this limitation of the system, this rather simplistic model has been challenged in recent times. With emergence of data on the multiple players involved in the cytokine regulation and the mechanism of acquired resistance to leishmaniasis, and in context of the newly discovered functions of Th3 and Th17 subsets of helper T-helper cells, further complexities are likely to be discovered.

4.3.1 **Th1 responses**

Control of a leishmanial infection requires the T-cell dependent activation of macrophages to attain a microbicidal state capable of restricting parasite replication. Experiments using various knockout mice have shown that the minimal requirement for effecting a cure in a leishmanial infection are the presence of CD4 T cells (Holaday et al., 1991; Moll et al., 1988; Varkila et al., 1993), peptide presentation on MHCII, Th1 inducer IL-12 and induction of Th1 cytokine IFN-γ (type 1 cytokines), activation of macrophage microbicidal enzyme iNOS and the resultant synthesis of NO. Consistent with this model of type 1 cytokine responses, mice deficient in type 1 cytokines, IL-12
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(Mattner et al., 1996) or IFN-γ (Wang et al., 1994), cytokine receptors such as IFN-γ receptor (Swihart et al., 1995), transcription factors such as T-bet (Szabo et al., 2002) or STAT-4 (Signal transducer and activator of transcription protein-4) (Stamm et al., 1999) and co-stimulatory molecule interactions CD40-CD40L (Campbell et al., 1996; Kamanaka et al., 1996), have all been shown to exhibit increased disease susceptibility.

IL-12 plays a central role in the orchestration of a curative, type 1 cytokine response. Myeloid cells including macrophages and dendritic cells are the major sources of IL-12 production, as proved by the inability of primed Th1 T cells from healed wild-type mice to transfer immunity to IL-12 deficient mice (Park et al., 2000). As mentioned previously, macrophages infected with Leishmania exhibit impaired IL-12 production, hence DCs including tissue resident Langerhan’s cells (LC), have been identified as the predominant IL-12 secreting cell type, both in vitro (Konecny et al., 1999; von Stebut et al., 1998) and in vivo (Belkaid et al., 2000; von Stebut et al., 2000). LCs have also been reported to transport Leishmania from the site of infection to the draining lymph node (Moll et al., 1993) leading to T- cell priming in the presence of secreted IL-12, thereby playing an early role in determining the eventual T-helper response. Natural killer (NK) cells have also been identified as sources of IL-12, but are not indispensable for induction of an anti-leishmanial Th1 response as demonstrated by efficient IL-12 dependent IFN-γ responses induced in CD4 T cells and efficient lesion resolution in NK cell deficient mice (Satoskar et al., 1999; Wakil et al., 1998). Naïve CD4 T cells have low levels of IL-12 receptor expression, which are upregulated on cognate TCR ligation. In addition, early secretion of IFN-γ by DCs and NK cells leads to activation of transcription factor T-bet, initiating a commitment of responding T cells towards a Th1 response and upregulation of IL-12 receptor (Afkarian et al., 2002; Lighvani et al., 2001). Upregulation of and
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signaling through the IL-12 receptor and IL-18 receptor, then activates transcription factor STAT-4 leading to commitment to Th1 responses (Ouyang et al., 1999; Yang et al., 1999). In addition to augmentation of IFN-γ secretion, both IFN-γ and IL-12 signaling pathways have been reported to participate in silencing of the Th2 locus (Manetti et al., 1993; Seder et al., 1993) by mediating suppression of key Th2 transcription factor GATA-3 (Ouyang et al., 2000), resulting in inhibition of IL-4 responses.

4.3.2 Th2, Th3 and Tr1 responses

Th2 lineage commitment is dependent on the induction of Th2 lineage transcription factor GATA-3 by the IL-4/STAT-6 signaling pathway (Kurata et al., 1999; Ouyang et al., 1998) and TCR-CD28 mediated GATA-3-independent pathway (Rodriguez-Palmero et al., 1999). The resultant IL-4 production results in diminished IFN-γ expression and stabilizes GATA-3 induction by chromatin remodeling (Lee et al., 2000; Ouyang et al., 2000). Lowered Th1 responses, eventually lead to inhibition of nitrite generation, resulting in enhanced parasite survival.

Activation of a distinct subset of IL-4 secreting CD4 T cells, possessing Vβ4Vα8 TCR recognizing the leishmanial LACK antigen (Leishmania homologue of receptors for activated C kinase), has been shown to be induced very early in infection in the BALB/c mice and was believed to confer disease susceptibility. This was based on the observation that infected Vβ4 deficient BALB/c mice mounted Th1 biased CD4 T cell response, resulting in improved parasite clearance (Himmelrich et al., 2000; Launois et al., 1997). Recent evidence has, however, demonstrated similar levels of activated LACK-CD4 T cells in resistant and susceptible mouse strains, early in infection (Stetson et al., 2002). Further, generation of Th2 cytokines in resistant mice by administering IL-4 (Chatelain et
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al., 1992) and anti-IL-12 antibodies (Hondowicz et al., 1997), early in infection, did not result in a sustained Th2 response and eventually still led to clinical cure.

Therefore, though IL-4 mediated Th2 response has been shown to result in non-healing leishmanial infection in mouse strains such as the BALB/c, these studies indicated that IL-4 was not the sole and sufficient factor regulating disease susceptibility, and that other cytokines such as IL-13, IL-10 and TGF-β (inducers of Th3 and Tr1 responses) might contribute in determining the disease phenotype. Studies using BALB/c mice deficient for IL-4 and IL-4-receptor-α chain (IL-4Ra) (Noben-Trauth et al., 1999) have reported partial improvement in the former strain and marked improvement in the later. However, mice with double deficiency of IL-4 and IL-13, whose receptor shares its α-chain with the IL-4-receptor, showed an even greater degree of resistance than either of the parental knockout mice (Matthews et al., 2000). TGF-β has also been reported to suppress Th1 responses and macrophage activation. Treatment of infected mice with anti-TGF-β antibodies has been reported to result in enhanced resolution of lesions, in spite of no observable effect on the levels of IFN-γ or IL-4. This improvement in clearance has been speculated to involve activation of macrophage effector molecules, leading to the enhanced levels of NO detected in the lesions after antibody treatment (Li et al., 1999). IL-10 has also been attributed with similar properties of Th1 and macrophage function suppression (Moore et al., 2001). IL-4Ra deficient mice show enhanced resistance on suppression of IL-10 function, indicating a role for IL-10 in conferring disease susceptibility (Noben-Trauth et al., 2003). Enhanced IL-10 levels have also been associated with human cases of chronic localized leishmaniasis, post-kala-azar dermal leishmaniasis (PKDL) and visceral leishmaniasis. IL-10 is produced by a wide variety of cells including macrophages, B cells, DCs and regulatory T cells (T-Regs), and the exact
source of IL-10 over-production in leishmanial infection has been a subject of much debate. Early studies have shown production of IL-10 on macrophages stimulated in vitro with LPS and opsonised *Leishmania*, resulting in suppression of IL-12 and TNF-α production (Kane and Mosser, 2001). However, as anti-CD4 treatment mediated depletion of CD4 T cells (Kropf et al., 1997) in IL-4 deficient mice BALB/c mice resulted in rapid control of infection, CD4 T cells were implicated as the crucial source for IL-10 in vivo. This view was further strengthened by the high levels of IL-4 and IL-10 mRNA expression levels reported in CD4 T cells from BALB/c mice (Reiner et al., 1994). IL-10 and Fcγ-receptor deficient mice have been reported to produce lower levels of IL-10 and show enhanced lesion resolution (Buxbaum and Scott, 2005). Recent studies have demonstrated production of IL-10 by both conventional CD25^-CD4 T cells and CD25^+CD4 regulatory-T cells in a mouse model of chronic leishmanial infection (Anderson et al., 2007; Jankovic et al., 2007). Further, antigen specific IFN-γ secreting Th1 cells at the lesion site were the major contributors to this IL-10 response, and depletion of the T-Reg population, led to exacerbated disease characterized by overproduction of Th2 cytokines including IL-10 (Anderson et al., 2007; Jankovic et al., 2007). These data suggest that antigen specific Th1 cells induced early in infection in the setting of a strong inflammatory response also participate in a feedback control of inflammatory responses in chronic infection, by secreting the IL-10 as a suppressive cytokine.

4.3.3 *Th17 responses*

Recent work has identified a novel T-helper subset of CD4 T cells (Th17) that are pro-inflammatory in nature and have been implicated in the pathogenesis of autoimmune
diseases. Th17 cells predominantly secrete IL-17 (hence referred to as Th17), in addition they also secrete TNF-α and IL-6 (Langrish et al., 2005). Th17 cells induce many pro-inflammatory cytokines and chemokines leading to tissue infiltration and uncontrolled inflammation. Antibody mediated blockade of IL-17 has been reported to improve the clinical outcome in adjuvant-induced arthritis (Bush et al., 2002) and experimental autoimmune encephalomyelitis (EAE)(Komiyama et al., 2006). Naive T cells activated in the presence of IL-6 and TGF-β have been shown to differentiate into Th17 T cells (Veldhoen et al., 2006). Further expansion of the Th17 response is thought to require the presence of an IL-12 family cytokine, IL-23. Several cytokines have been reported to enhance or attenuate Th17 differentiation or expansion (Bettelli et al., 2007). Cytokines such as IL-1 and TNF, and co-stimulatory molecules ICOS (inducible co-simulator)(Park et al., 2005) and OX40 (Nakae et al., 2003) enhance the generation of Th17 cells. IL-27 expression, on the other hand, negatively regulates Th17 differentiation (Hunter, 2005). The differentiation of Th17 cells is independent of Th1 and Th2 transcription factors, STAT-4 and STAT-6 (Park et al., 2005), and has been identified to be regulated by a separate transcription factor, RORγt (Akimzhanov et al., 2007). Th17 T cells have now been implicated in disease exacerbation in various autoimmune diseases, previously believed to be mediated by Th1 cells (Langrish et al., 2005; Nakae et al., 2003). Ironically, the Th1 cells in such diseases have now been shown to be involved in disease protection due to the inhibitory effect of IFN-γ on Th17 induction (Krakowski and Owens, 1996; Park et al., 2005; Tran et al., 2000; Willenborg et al., 1996). Further, IFN-γ, IL-27 mediated Th17 suppression requires active signaling through a STAT-1 mediated pathway (Batten et al., 2006).
Recent correlational studies on the lesion progression and parasite persistence using the Leishmania amazonensis (L \textit{amaz}) model of infection indicate interesting possibilities on the modulatory effects of these cytokines. The Lm resistant C57BL/6 mice have been reported to be sensitive to \textit{L amaz} induced cutaneous leishmaniasis, characterized by progressive lesions in the presence of a robust Th1 response, and the lack of IL-4 over-production (Ji et al., 2002). A recent study using two strains resistant (TR) and susceptible (TS) to induction of oral tolerance, has shown increased disease susceptibility in the TR but not in the TS strain on \textit{L amaz} infection (Tavares et al., 2006). Cytokine analysis showed low IL-10 and TGF-\(\beta\) responses, greater lesion size and a paradoxically low lesion parasite burden in the TR mice, with the TS mice showing the converse phenotype. On induction of oral tolerance with low dose of antigen, TR mice showed a regression of lesion and a surprising exacerbation of lesion on immunization with high dose of antigen. The TS strain did not reveal any modification of the phenotype on immunization with either dose of antigen. In view of the known role of IL-10 as an immunosuppressive cytokine, it is possible that the exacerbated lesions seen in the TR strain could be due to the lack of this cytokine, and conversely, higher IL-10 levels in the TS strain could explain lower lesion size and increased parasite persistence in accordance with the NO suppression induced by IL-10. Since induction of oral tolerance leads to increase in the levels of suppressive cytokines IL-10 and TGF-\(\beta\) (Weiner et al., 1994), this could be a reason for the decreased lesion size seen in TR mice immunized at lower antigen doses. Paradoxically, the higher TGF-\(\beta\) levels, could also have led to the induction of Th17 responses at the higher antigen dose, resulting in further exacerbation of disease. Though the role of IL-17 producing T cells in leishmanial infections has not yet been properly addressed, it is likely to play a role in acute exacerbations in disease
after long periods of latency as seen in human cases of PKDL and mucocutaneous leishmaniasis, possibly brought on by a compromise in the IL-17 inhibitory IFN-γ/Th1 responses. Studies to dissect the possibility of the involvement of such operant mechanisms could help better understand human clinical disease exacerbations.

5. Immune modulations by retroviruses

Viruses have been long characterized to induce immune response modulations to improve their infectivity as well as survival. Modulations mediated by retroviruses have come into focus due to the HIV (human immunodeficiency virus) mediated AIDS (acquired immunodeficiency syndrome) pandemic. Other retroviruses, including MMTV (murine mammary tumor viruses) have also been extensively studied and characterized to modulate host immune responses. Retroviruses, integrate into the host genome to gain replication sufficiency, and in the process pose the inherent risk of not only causing modulations in host gene responses, but in the event of inactivating mutations to the viral template the integrant replication deficient viruses, gain vertical transmission and continue to mediate effects on the host genome across subsequent generations.

HIV viral protein Nef (negative factor) participates in down-regulation of surface MHC (Cohen et al., 1999; Stumptner-Cuvelette et al., 2001) and co-stimulatory molecules (Chaudhry et al., 2005) to prevent recognition of virus infected cells by the immune system. In addition, Nef induces secretion of inflammatory cytokines to cause recruitment and activation of T cells (Swingler et al., 1999), which are otherwise resistant to infection in the resting state, thereby facilitating its transmission to uninfected cells. MMTVs, members of beta-retrovirus family, infect mammary tissue in female mice and
are undergo milk borne vertical transmission to the pups, where they track to the
intestinal lymphoid tissue to infect B lymphocytes and dendritic cells (Baribaud et al.,
1999; Golovkina et al., 1999). The v-SAg (viral encoded superantigen) then binds to
MHCII on these infected cells, resulting in activation of large populations of CD4 T cells
due to ligation of particular TCR-variable-β regions (TCR Vβ)(Janeway, 1991). The
cytokines secreted by these CD4 cells leads to activation of bystander cells, which then
become susceptible to MMTV infection, in a similar fashion to that observed with HIV
(Baribaud et al., 1999; Maillard et al., 1998). At later stage in infection, these v-SAg

Some of these retroviruses incur inactivating mutations, rendering them replication
deficient, and remain genome integrant. Such genome integrant retroviruses have been
estimated to occupy almost 8% of the human genome and up to 6-8 MMTV integrant
proviral sequences (mtv) have been identified in most inbred mouse colonies (Kozak et
al., 1987). Some of these mtv sequences however retain their ability to encode for SAgs,
which now mediate complete thymic deletion of reactive subsets, rendering the host T
cell repertoire deficient in certain TCR Vβ subsets, and since this deletion occurs at
negative selection, it affects both the CD4 and CD8 T cell compartments (Tomonari et al.,
1993). Such a loss of TCR Vβ subset, mediated by the mtv-7 locus, has been associated
with conferring resistance to cerebral malaria in mice due to the deletion of the causal Vβ
8.1 subset (Gorgette et al., 2002). MMTV integrations have also been reported to mediate
susceptibility to tumors (Scianimanico et al., 1999), graft-versus host disease (GVHD)
(Allen et al., 2000) and bacterial infections with Vibrio cholerae (Bhadra et al., 2006),
and in all these instances, MMTV integrations have been reported to increase disease
susceptibility independent of their T cell deletion function. Studies on a model of GVHD
with \( mtv-7 \) integrations have demonstrated that the development of disease was independent of \( mtv-7 \) mediated deletion, and have speculated the role of it’s modulation on surface so-stimulatory molecules, CD48 (a CD2 ligand involved in regulating the Th1/Th2 balance) in this case, which are in linkage with the \( mtv-7 \) locus (Allen et al., 2000). In another study, an \( mtv \)-null mouse model was found to be resistant to both MMTV and \( V \) cholerae infections, both of which did not posses any shared antigenic epitopes (Bhadra et al., 2006). Further, susceptibility to both infections could be reconstituted in the \( mtv \)-null mice by an endogenous provirus lacking the coding sequences for the immunodominant SAg protein (Haak-Frendscho et al., 1994). Therefore, these data appear to be suggesting a deletion function independent mechanism for immune modulation by such proviral integrations.

Many of the retroviral integrants are believed to have been positively selected during evolution due to their ability to protect against viral infections as demonstrated in recent study where changes in an immune protein have been shown to confer protection against ancient retroviruses which presumably plagued our primate ancestors (Kaiser et al., 2007). On the flip side, however, these very changes might have rendered us susceptible to newer retroviruses, including HIV (Kaiser et al., 2007), or other MMTVs in case of mice (Bhadra et al., 2006). Reactivation of ancient retroviruses is a further possibility arising out of recent studies which demonstrated this possibility using surrogate viral sequences to restore transcriptional activity and replication competency in integrant replication deficient proviruses (Lee and Bieniasz, 2007; Stauffer et al., 2001; Sutkowski et al., 2001).