Introduction
Adaptive immune responses are mediated by the T and the B lymphocytes with help from dendritic cells (DCs) and macrophages and are characterized by features such as antigenic memory, clonal diversity and stringent self-non self discrimination. Both T and B lymphocytes express clonotypic receptors on their surface for specific recognition of target antigen. B cell receptors (BCR) can recognize specific antigenic epitopes directly on an intact circulating antigen while T cell receptors (TCR) recognize antigen that has been processed into peptides and presented in association with molecules of the major histocompatibility complex (MHC). The receptors are generated by germ-line gene rearrangements in the bone marrow that results in a diverse repertoire of clones, a vital strategy adopted by the immune system to fight the extremely high diversity observed in pathogenic organisms. During development these receptors get screened for their reactivity against self and only those clones which do not bind self antigens with high affinity survive over time and constitute the peripheral pool of lymphocytes. To accommodate the huge repertoire of lymphocytes within the limited space of the lymphoid tissue, each of the clones is represented in very small numbers in the periphery. Thus one crucial step following engagement of the receptors by cognate antigen is the triggering of the antigen specific clones for expansion. Clonal expansion is followed by differentiation either into relatively short-lived effector cells that help in the immediate clearance of infection, or into resting memory cells which survive for longer periods of time and can respond to a subsequent antigen encounter by rapid proliferation and differentiation.

The BCR is a complex which consists of an antigen binding membrane immunoglobulin (mlg) that is non-covalently associated with two polypeptide chains, CD79a and CD79b, which are responsible for initiation of downstream signaling (Gauld et al., 2002; Harwood
Ligation of the BCR by its cognate antigen is followed by phosphorylation of certain motifs in the cytoplasmic tails of the CD79a and the CD79b molecules, called the immuno-tyrosine receptor activation motifs or the ITAMs. Phosphorylation of these ITAMs results in formation of a signaling complex which contains 3 classes of activated protein tyrosine kinases (the Src family kinase Lyn, the Syk kinase, and the Tec family kinase Btk) and various adaptor molecules. The downstream events include mitogen activated protein kinase (MAPK) activation and increase in intracellular Ca\(^{2+}\) levels, which eventually lead to the activation and subsequent nuclear translocation of transcription factors, NF-kB and NF-AT, as well as to the activation of other factors like c-jun and fos, that alter their DNA binding ability. B cell activation is an outcome of signals transduced through the BCR along with signals generated from certain co-receptors such as, CD19, CD21, CD22, CD32 and CD72 that are expressed on the B cell surface and can either positively or negatively modulate BCR signal transduction (Danzer et al., 2003; Ono et al., 1997; Shoham et al., 2006; Tedder, 1998).

Apart from recognition of its target through the BCR, B cells can also employ other receptors, such as the toll-like receptors (TLR) for recognizing pathogen associated molecular patterns and undergo activation. For example, binding of lipopolysaccharide (LPS) to TLR4 on B cells can activate B cells resulting in its proliferation, differentiation into plasma cells and death in a T-independent (TI) fashion (Akira and Takeda, 2004; Fagarasan and Honjo, 2000). Plasma cells represent the final stage of B cell differentiation and are devoted to the production of large amounts of Ig. Another class of TI antigen are generally repeating polymers like dextran, present on the surface of the pathogens, which can crosslink a number of BCRs on a B cell surface resulting in B cell activation. The TI
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responses usually take place in the marginal zone of the spleen, but TI antigens can also stimulate B cells at other locations, such as, in the peritoneal cavity (Fagarasan and Honjo, 2000; McHeyzer-Williams, 2003). The plasma cells in case of a TI response have short half-lives and can secrete IgM and low levels of IgG1 and IgG3. There is little affinity maturation of antibodies or generation of antigenic memory in case of such responses (Fagarasan and Honjo, 2000; Macpherson et al., 2000; McHeyzer-Williams, 2003; Montecino-Rodriguez and Dorshkind, 2006).

For eliciting effective humoral responses to most protein antigens, a B cell requires help from antigen specific T cells. Such antigens are referred to as T-dependent (TD) antigens. The T cell help may be either in the form of cognate cell to cell interactions or in the form of cytokines secreted by the T cells. During a TD immune response, naïve B cells carrying antigen specific receptors are activated within the extra-follicular T cell areas of the secondary lymphoid organs and initiate formation of foci of proliferating B cells. Here, they can undergo class switching and show inter-clonal competition due to differences in their relative affinity for the antigen and undergo differentiation into plasma cells with short half-lives (Liu et al., 1991; Slifka et al., 1998; Toellner et al., 1996). A few B cells do not undergo terminal differentiation in the foci, but move into the follicles where they interact with CD4 T cells and follicular dendritic cells (FDCs) to establish germinal centres (GCs) (Klein and Dalla-Favera, 2008). In the GCs, the B cells undergo extensive proliferation coupled to somatic hypermutation in the form of random mutations introduced in the variable region of the BCR, producing receptors with varying affinities for the antigen (Papavasiliou and Schatz, 2000; Wagner and Neuberger, 1996). A significant proportion of these B cells undergo apoptosis while competing for the limited amount of antigen
presented on the FDCs in the GCs and only the B cells expressing BCRs with high affinity for the antigen are selected over time. Such affinity-based selection of responding B cells over time, especially under steadily decreasing amounts of antigen in the milieu result in affinity maturation of the antibody response when the mutated B cells differentiate into plasma cells (Han et al., 1997). Class switching also takes place in the GCs where there is switching of isotype from IgM to any of the downstream isotypes, such as, IgG, IgA or IgE, that have diverse physiological functions. As only a few B cells from the extrafollicular foci form the founder GC B cells, there is majorly an intra-clonal competition among the B cells in the GCs. The selected high affinity clones then exit the cell-cycle and undergo differentiation either into memory B cells or into plasma cells. Some of the plasma cells that find niche in the bone marrow are retained there and can continue secreting antibodies for long periods of time (Arpin et al., 1995).

A B cell needs to present antigen derived peptides in association with MHCII molecules in order to recruit help from antigen specific T cells. B cells along with DCs and macrophages are known as professional antigen presenting cells (APCs) that can provide continual surveillance against extracellular pathogens and present pathogen derived peptides to CD4 T cells in the context of MHC class II molecules. Unlike macrophages and dendritic cells, B cells are relatively inefficient at uptake of antigens through phagocytosis or pinocytosis (Trombetta and Mellman, 2005; Watts, 1997). They are, however, highly efficient at internalizing antigens through their surface BCR into endosomes. Internalized antigens move through the different compartments of the endocytic pathway before they reach the lysosomes where receptor antigen uncoupling and antigen degradation occur. In APCs, there are specialized compartments known as the MHC class II containing compartments (MIICs)
that have late endosomal / lysosomal characteristics (Neefjes, 1999; Stern et al., 2006; Tulp et al., 1994) and are highly efficient at processing antigens and generating peptide MHCII complexes (pMHCII). The efficient sorting of the internalized BCR to early endosomal and late endosomal compartments requires association of the BCR with certain adaptor proteins and kinases like BLNK and Syk respectively, that have been known to play a role in BCR signal transduction (Lankar et al., 1998; Siemasko and Clark, 2001; Siemasko et al., 2002). The decreasing pH and the presence of several proteases in the endocytic compartments helps in degradation of the antigen and generation of peptide fragments of the exact size that can fit into the peptide binding groove of the MHC II molecules (Trombetta and Mellman, 2005; Watts, 1997).

The peptide binding groove in MHCII molecules remain occupied by the invariant chain (Ii) when the newly synthesized molecule moves from the trans-golgi network to the endocytic pathway (Cresswell, 1994; Kvist et al., 1982). Sequential degradation of Ii in the endocytic compartments finally leaves a small fragment that stays associated with the peptide binding groove of the MHCII molecule and is called the class II associated invariant chain peptide (CLIP). Removal of CLIP and loading of peptide fragments onto MHCII molecules involves a chaperone protein called H-2M (Alfonso and Karlsson, 2000; Karlsson, 2005; Miyazaki et al., 1996; Morris et al., 1994). Another chaperone H-2O, present in B cells is known to inhibit the function of H-2M in a pH dependent manner (Alfonso et al., 2003a; Alfonso et al., 2003b) with the inhibitory effect being greatest at pH above 5.5. Thus, in B cells this favours presentation of antigens from the late endosomal and lysosomal compartments. The pMHCII complexes transported to the surface of the B cells can then recruit antigen specific T cell help. The initial contact between a T cell and a B cell
is antigen independent and depends on adhesion mediated by ICAM-1/LFA-1 and CD2/CD48 molecules while the TCR scans the peptide-MHC complexes on the B cell surface (Lee et al., 2002). Following establishment of antigen specific contact, members of the B7 family of receptors like CD80, CD86 (Azuma et al., 1993; Chambers and Allison, 1997; Kariv et al., 1996; Linsley and Ledbetter, 1993), ICOS (McAdam et al., 2000), PD-L1 (Freeman et al., 1989), PD-L2 (Latchman et al., 2001) as well as the tumor necrosis family receptors on the B cells like CD40 (Grewal and Flavell, 1998), CD27 (Akiba et al., 1998; Kobata et al., 1995), CD30 (Cerutti et al., 2000; Cerutti et al., 1998), and CD95 (Catlett and Bishop, 1999; Krammer, 2000) are known to modify the T cell – B cell interactions (Bishop and Hostager, 2001) and modulate B cell proliferation, class switching, somatic hypermutation, differentiation and death.

There are two factors that regulate the outcome of B cell activation, namely, the strength and duration of the signal generated from the BCR and the quality of the T cell help available. To dissect which of these factors play a dominant role in controlling different aspects of B cell activation, we used the Beige (bg/bg) mouse. This is a mouse homologue for the Chediak-Higashi syndrome (CHS) in humans with mutation in the lysosomal trafficking regulator (lyst) gene (Barbosa et al., 1996; Shiflett et al., 2002; Spritz, 1998). LYST has been shown to be associated with microtubules (Faigle et al., 1998) and exhibits a cytosolic distribution (Perou et al., 1997). Mutation in this gene affects lysosomal morphology and functioning in many cell types like the melanosomes, platelets, cytotoxic T cells, natural killer cells and neutrophils (Baca et al., 1989; Bahadoran et al., 2001; Haliotis et al., 1980; Paigen et al., 1990; Salles et al., 2008). Efficient functioning of the endosomal / lysosomal pathway is an important parameter that will determine the time for which a BCR
ligand complex remains intact before it gets degraded in the lysosomes. Since it is known that the BCR remains associated with different signaling intermediates while it gets sorted to the compartments of the endocytic pathway, it is possible that the BCR may retain its signaling potency in the early endosomes and that in bg/bg B cells the signaling competence may be maintained for a longer period of time as compared to wild type (WT) B cells. The other outcome of a defective endo-lysosomal pathway might be the inability of B cells to efficiently process and present antigenic peptides to T cells. In support of this hypothesis, B cells from CHS patients show delayed peptide loading onto MHCII molecules as well as a delay in the transport of the pMHCII complexes to the cell surface (Faigle et al., 1998). These may translate into decreased or delayed responsiveness on the part of the T cells.

Thus, this study has attempted to assess the effect of lyst mutation on B cell antigen presentation following antigen uptake through different endocytic routes and the duration of BCR signaling following its internalization. Since the strength and duration of signaling from the BCR and the ability of B cells to present antigens to T cells, both contribute towards modulating B cell responses, we used bg/bg mice as a tool to dissect the relative contribution of these two factors in regulating humoral immune responses.