RATIONALE FOR THE PRESENT STUDY
In a T-dependent humoral response, exposure to an antigen activates a complex machinery of events that leads to the generation of an effector response to antigen. Various parameters influence the development of a humoral response against a given antigen. These are - the concentration of antigen, the duration and timing of the antigen encounter, the avidity with which the antigen binds to B cells, and the interaction of B cells with other lymphocytes. Depending upon the variations in the above factors, B cells can generate both qualitatively and quantitatively distinct responses. The induction of a T-dependent humoral immune response begins with the specific interaction between the BCR and antigen. BCR crosslinking by antigen leads to activation of resting B cells. This is mediated by the recruitment of several signaling pathways (Campbell, 1998; Defranco, 1997). The diversity and crosstalk among these pathways then regulate the outcome of the humoral response (Kurosaki, 1997; Benschop and Cambier, 1999).

Antigen dependent activation of B cells first occurs in the extrafollicular sites in the T cell rich area. The majority of activated B cells within the PALS differentiate to foci of antibody forming cells (AFCs). AFCs secrete low affinity unmutated IgM and IgG class of antibodies for 10-12 days after primary immunization and, subsequently die of apoptosis (Jacob et al., 1991; Jacob and Kelsoe, 1992).

A small proportion of antigen activated B cells migrate from the PALS to the primary follicles to seed germinal center (GCs), which later resolves into a light and a dark zone. The number and size of GCs increase with the concomitant decrease in the number of foci. Proliferating B cells within the germinal centers undergo somatic hypermutation in their immunoglobulin variable region genes (Jacob et al., 1991; Jacob and Kelsoe, 1992). This is accompanied by affinity driven selection and specificity maturation (Berek and Milstein, 1987; Berek, 1994). Finally antigen specific mutant B cells differentiate into either plasma B cells, or, memory B cells.

Based on the clonal relatedness between the B cells within the two compartments, a consensus view has emerged in the literature, which propose that the cells within the two cellular compartments are clonally related and derived from a common precursor. Thus, it is a subset of B cells, which gets activated in the T cell rich area by T dependent antigen and form foci, that is thought to migrate into the follicle to initiate the GC reaction (Jacob and Kelsoe, 1992). Although the clonal relatedness of cells which seed
Rationale for the present study

GCs or AFCs is generally accepted an alternate explanation has also been put forward. It was suggested by Klinman that activated B cells which populate GCs, and those that form AFCs are derived from different precursor cells which may represent distinct cellular lineages (Linton et al., 1989; Linton et al., 1992; Decker et al., 1995). These populations of the cells were distinguishable on the basis of expression of the heat stable surface antigen CD24. It was demonstrated that only the CD24 low subpopulation of cells was capable of seeding GCs, whereas high CD24 B cells differentiated into foci (Linton et al., 1992).

The cells within the two compartments also differ phenotypically and can be distinguished on the basis of binding to plant lectin – PNA (Rose et al., 1980). Recent evidence suggests that some degree of heterogeneity exists between these two subsets of cells. A number of studies have shown that the overall clonal heterogeneity in GC B cells is decreased when compared with those that are initiated in PALS (Liu, 1997; Kelsoe, 2000). Thus, not all antigen activated B cells enter the follicle and proliferate to initiate the GC reaction. Rather they are subjected to a stringent selection process which operate at pre GC phase.

Using a synthetic peptide model, earlier studies from our laboratory have shown that the decision-making process, i.e. whether an antigen activated B cell will form AFC or develop into the GC is governed by the affinity of the BCR- antigen interaction. In these studies the T-dependent model antigen used was a synthetic 38 amino acid residue polypeptide – PS1CT3 (HQLDPAFGANSTNPDDGIEKKKIAXMKASSVFNVVNS). This peptide was a chimeric peptide in which residue 1-15 (segment PS1) represented a B cell epitope derived from the pre-S1 sequence of the surface antigen of hepatitis B virus (Neurath and Kent, 1988). The carboxy- terminal 21 residues (segment CT3) corresponded to the promiscuous T cell epitope derived from the circumsporozoite protein of the malaria parasite Plasmodium falciparum (Sinigaglia et al., 1988). Separating the B and T cell epitopes was a spacer of two glycine residues at position 16 and 17 (Rao and Nayak, 1990). It was found that the initial encounter of preimmune B cells with the PS1CT3 induced a primary IgM response that was directed against all accessible domains on the PS1 segment (Agarwal et al., 1996). These early activated clonotypes were soon subjected to a stringent selection process that ensured survival of
only high affinity B cells (Agarwal et al., 1996). This selection step was shown to occur prior to GC selection, and was enforced by the need for the diverse Ag-activated B cells to compete for a limiting pool of Ag-primed T helper cells (Agarwal and Rao, 1997). Further, it was demonstrated that while the Ag-driven activation of preimmune B cells could be achieved in the presence of marginal levels of T cell help, subsequent generation of the IgG response required significantly higher numbers of Ag-specific Th cells. It has also been shown that besides their requirement for lower levels of T cell help, perimmune B cell activation could be induced by very low affinity interactions (Ka ≥10^4 M^-1) with antigen (Nakra et al., 2000). These collective results, therefore, suggested that antigen dependent activation of naive, resting B cells was a facile process requiring low activation thresholds. Both limited frequency of Ag-primed Th cells and low affinity antigen BCR interactions could achieve this. As opposed to this, seeding of GCs was found to be restricted to only that subset of activated B cells that displayed a high affinity for antigen binding (Agarwal et al., 1998). Therefore, the potential of an antigen activated B cell to seed GC appears to be governed by the affinity of the BCR.

These studies raised the probability that the affinity of antigen for the BCR may constitute the regulatory parameter that defines the extent of GC formation in a primary humoral response. The present study, therefore, was undertaken to identify at least some of the underlying mechanisms by which affinity of BCR for antigen may regulate the functional status of preimmune B cells. The succeeding chapters describe my results in this direction.