SUMMARY
Immunization with a T-dependent antigen leads to activation of B cells, following which, foci of specific Ab-producing cells are found to concentrate in the periphery of the PALS (Jacob and Kelsoe, 1992; MacLennan, 1994). This activation is characterized by several cell surface phenotypic changes which include the downregulation of sIgD receptors, and increased levels of CD86, CD69, and the MHC class II molecules (Mond et al., 1981; Noelle et al., 1986; George and Claffin, 1992; Lenschow et al., 1994). Within the next few days, GCs can be observed to develop within the primary B cell follicle, where, processes related to affinity maturation and B lymphocyte differentiation into either memory or plasmacytes are initiated (Kosko-Vilbois et al., 1997; Kelsoe, 1995).

Although a clonal relatedness between cells in the AFCs and those in GCs has been established, it, however, appears that not all antigen-activated cells can seed GCs. Rather, cells that populate GCs have been shown to represent the survivors of a stringent filtering process where selection occurs on the basis of the affinity of individual clonotypes for antigen. In other words, an affinity threshold barrier operates in the pre-GC phase which restricts entry of only the high affinity B cells to the follicular compartment (Agarwal et al., 1998). Those B cells which are competent to seed GCs differ from antigen activated B cells in the additional expression of an array of cell surface molecules. Some of these include PNA-R, CD24, CD95, and the activation marker GL7 (Rose et al., 1980; Hardy et al., 1984; Lalor et al., 1992; Han et al., 1997; Kimota et al., 1997; Lahvis and Cerny, 1997; Koni et al., 1999). Although parameters which regulate the ability of antigen activated B cells to seed GCs are not yet fully understood, several studies have demonstrated an obligatory role for specific transcription factors such as Bob-1/OCA-B, expression of a chemokine receptor BLR-1, and costimulatory molecules such as CD40 and those belonging to the B7 family (Schubart et al., 1996; Forster et al., 1996; Foy et al., 1994; Ferguson et al., 1996).

From our earlier findings that the antigen-binding affinities represents at least one important parameter that defines the GC seeding competency of primary B cells, it seemed reasonable to assume that both qualitative and quantitative variations in the nature of BCR cross-linking may constitute the underlying factor responsible for this effect. The present studies, therefore, were undertaken to explore this aspect.
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From the standpoint of functional relevance we distinguished, in our studies, between two separate states of activation of resting B cells. One of these represented the generation of cells expressing only an activated phenotype (i.e. sIgD<sup>lo</sup>, CD86<sup>hi</sup>, I-A<sup>hi</sup>), whereas the other identified cells which — in addition to the activation markers — also expressed surface markers that were typical of GC B cells (PNA-R, CD24, and GL7). We anticipated that such a distinction may help to discern between conditions that only drive activation, versus those that also confer on the target cells the ability to seed GCs. This segregation into two states of activation was further supported by our experiments demonstrating that the ability of anti-IgD activated B cells to populate GCs was contingent upon their expression of at least the GC markers that were studied.

An important aspect of our initial experiments was the identification of kinetic distinctions between the two states of activation, at least in response to stimulation with anti-IgD. Thus, while phenotypic alterations characteristic of activated cells could be achieved upon stimulation with anti-IgD for only 24 h, optimal expression of all the GC markers required a stimulation period of as much as 60 h. This latter observation was consistent with our findings that establishment of peak GC seeding efficiency also required such a prolonged duration of anti-IgD stimulation.

Our subsequent experiments clearly revealed that the duration of BCR-triggering constitutes at least one regulatory factor responsible for the differing rates of induction of activation-related, and GC markers. Thus, whereas sIgD cross-linking times of only few minutes was sufficient to generate activated B cells, induction of the GC phenotype — and also the GC seeding potential — required sustained stimulation extending over several hours. These results, therefore, highlight the importance of the duration for which antigen is available in defining the extent to which specific resting B cells are activated. From a physiological point of view, therefore, the stability of antigen <i>in vivo</i> is likely to influence the outcome of a primary humoral response, particularly in terms of the magnitude of the GC response that is obtained.

In addition to <i>in vivo</i> stability, the amount of available antigen was also found to represent an additional regulatory parameter. Thus, while limiting levels were sufficient to produce an activated phenotype, induction of the GC phenotype — however — required significantly higher antigen concentrations. The variable effects of antigen concentration...
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reflect, in all probability, the consequences of the resulting variability in receptor occupancy. This, in turn, would suggest that BCR occupancy thresholds define the distinction between an activated and a GC phenotype. If this is indeed found to be true, the affinity of antigen for the BCR can then be also expected to exercise similar influences. Thus, by increasing receptor occupancy levels, high affinity interactions would favor generation of cells that are competent to seed GCs, whereas low affinity interactions would restrict to the production of cells with only an activated phenotype.

Our findings are also consistent with the observations of Nemazee et al. (Kauskoff et al., 1998), who demonstrated that high affinity antigens could initiate productive B-cell responses even at low concentrations, whereas moderate to low affinity antigens could achieve this only under conditions of increased BCR occupancy.

Although occasional exceptions have been noted (Lentz and Manser, 2001; Garcia de Vinuesa et al. 2000), the development of GCs is a phenomenon that is restricted to T-dependent humoral responses. This aspect was also evident in our present studies. Thus, while optimal concentrations of anti-IgD alone was sufficient to induce the GC phenotype from resting B cells, development of GCs from this population – however – was strictly dependent upon the presence of primed T cells in the hosts. Therefore, it became necessary to examine whether T cell-derived signals could potentially contribute towards regulation of the activation status of resting B cells.

While a cognate engagement of T by B cells involves a multitude of cell surface interactions (Bishop and Hostager, 2001; Tarlinton, 1998) we were forced, from practical considerations, to restrict the scope of our examination to those involving only three of the molecules expressed by B cells. Important insights could, nonetheless, be obtained from our findings that crosslinking of these surface molecules could potentiate responses to a limiting concentration of anti-IgD. Importantly, a combination of all three antibodies – anti-I-A, anti-CD40, and anti-CD54 – was required to achieve this. This suggests that it is the cooperative (or, additive) contributions from the multiplicity of interactions, established at the B-T interface, that is capable of overcoming the activation threshold barrier inherent to BCR-dependent pathways. The functional significance of these findings lies in the implication that BCR-dependent thresholds may become redundant, in cases where the antigen is able to efficiently prime T cells. This, in turn, may explain the
recent observations of Dalparto et al. (Dalparto et al., 2002) that – in the absence of their high affinity counterparts – low affinity B cells were also capable of populating GCs.

In summary, our results identify that the generation of an activated versus a GC phenotype from resting B cells occurs at independent rates, and involves distinct activation thresholds. Further, they also highlight that the magnitude of the GC response obtained from primary B cells is ultimately determined by a composite of parameters. At one level, a BCR-dependent threshold barrier regulates this where the stability and either concentration or affinity of antigen comprise the limiting factors. Importantly, this threshold does not constitute an invariant barrier and can potentially be overcome by contributions from antigen-specific T cells. In this case, however, the availability of primed T cells in the early stages of activation may prove to be essential since, as we have shown earlier (Agarwal and Rao, 1997), the GC seeding competency of primary B cells is established within forty-eight hours of immunization of mice with a T-dependent antigen (Agarwal et al., 1998). Consequently, the efficiency with which a given antigen can prime T cells may represent an important criterion that determines the extent to which these latter cells can contribute towards induction of the GC phenotype.

From a physiological standpoint, BCR-dependent thresholds are likely to be important in ensuring that only the high affinity subset, from the pool of antigen-activated B cell clonotypes, is recruited within GCs. As opposed to this, the regulatory contributions from primed T cells may serve by ensuring that antibody generation is not restricted only to the dominant antigens expressed by a given pathogen. Our present results, therefore, provide additional insights into processes that modulate the development of a T-dependent humoral response. It would, however, be of interest to elucidate the intracellular signals that mediate between the distinct BCR-dependent thresholds, and also how they interface with those that are contributed by the T cell-dependent stimuli.
A remarkable property of the adaptive immune system is its ability to recognize the multitude of foreign antigens on the one hand, and generate an exquisitely specific response on the other. However, the individual stages that regulate the generation of such an ordered and controlled response remains to be completely elucidated. A good example how regulatory processes govern the biological outcome is provided by the humoral immune response. Development of an effective humoral response depends upon a range of factors, one of which is the availability of antigen. Antigen availability is itself dependent upon the variety of factors - such as amount of antigen that has entered the body, in-vivo stability of the antigen, and also the effectiveness with which it interacts with B cells. Further, the efficiency with which B cells interact with the antigen is governed by the affinity of interaction, which in turn controls the degree of receptor occupancy. Depending upon the extent of variability of the above parameters, B cells generate a humoral response that is both qualitatively and quantitatively distinct (Goodnow, 1996). As already discussed in earlier chapters, these variabilities arise due to the resultant differences in the nature and strength of the BCR-dependent intracellular signals produced.

A humoral immune response involves the activation of appropriate B cell with the antigen, and the eventual formation of antibodies. These antibody molecules play a central role by binding to pathogens and then recruiting effector mechanisms to destroy the invaders. A humoral response can be subdivided into the primary and secondary humoral immune response depending upon the type of B cell that is activated. The initial encounter of a naïve B cell with an antigen induces a primary response. In this stage preimmune B cells – following antigen encounter- proliferate with the help of T-helper cells and subsequently differentiate into either antibody forming cells (AFCs) or memory B cells (Liu, 1997). AFCs start secreting antibody of IgM and IgG class. AFCs in the primary response do not undergo somatic hypermutation and affinity maturation and, therefore, secrete low affinity antibody. A lag phase of 5-7 days exists in the primary humoral response before antibody levels start increasing. This lag period is required for the activation of B cells with antigen, interaction with T-helper cells, and subsequent proliferation and differentiation into plasma cells. These low affinity antibodies form the immune complex with the antigen, are trapped within the follicular
dendritic cell (FDC) network. These immune complexes stay there for long periods and are responsible for the persistent activation of the memory B cells (Jacob and Kelsoe, 1991; Jacob and Kelsoe, 1992; MacLennan, 1994).

Because AFCs have short half lives, long lived antibody production depends upon the generation of memory B cells. The latter occurs within the germinal centers. Memory B cells can be distinguished from the preimmune B cells by their surface expression of Ig isotypes other than IgM and IgD. These include sIgG, sIgE, and sIgA. B cells, selected in the GCs to become memory cells, initiate the secondary humoral response upon a subsequent-encounter with the same antigen. These memory cells respond to antigen rapidly and generate a large number of plasma cells. These plasma cells secrete antibodies within 1-2 days of secondary infection and the antibody levels are between 100-1000 fold higher. In addition to this, the antibodies produced in a secondary response are of high affinity because of the high mutation rates associated with the memory B cells. Thus, the generation of memory cells that is responsible for the long lived antibody response and the subsequent eradication of pathogen is a critical step during the progression of a humoral immune response. A prerequisite for the generation of memory B cells is the formation of germinal centers. These are histologically defined areas formed within the secondary lymphoid organs.

As discussed in earlier chapters, initiation of humoral immune response occurs following a BCR – antigen interaction, which leads to activation of resting B cells. Each B cell displays approximately $10^5$ antigen receptors, all with a unique specificity, on its surface. This unique specificity is generated by random rearrangement of a series of gene segments encoding the Ig molecule. Thus each mature B cell possesses a functional gene encoding the immunoglobulin heavy chain and a single functional gene encoding the immunoglobulin light chain. In other words, each B cell synthesizes and expresses Ig molecules with a single specificity on its membrane (Alt, 1987; Burnett, 1959; Boehmer, 1994; Rajewsky et al., 1987). Activation of B cells with the antigen first occurs in the T-cell rich extrafollicular sites of secondary lymphoid organs. Subsequent to this, activated B cells follow two distinct and compartmentalized pathways of differentiation. They either form the foci of antibody secreting cells in the periphery of PALS, or migrate to the follicles to seed GCs. Antigen activated B cells in the foci differentiate to produce
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unmutated IgM and IgG class of antibody for 10-12 days and subsequently die of apoptosis. A few antigen activated B cells migrate from the PALS to the primary follicle and start proliferating with the help of T cells. These B cells gradually develop into the germinal centers where they undergo somatic hypermutation of immunoglobulin B cells. The mutated progeny undergo affinity and specificity maturation (Jacob et al., 1991; Jacob and Kelsoe, 1992; Berek et al., 1991, Berek and Zeigner, 1993). Finally antigen-specific mutant B cells bearing high affinity receptors are positively selected to differentiate into plasma and memory B cells (Klaus et al., 1980; Kosco et al., 1989; Pfeffer and Mak, 1994, ManLennan, 1994). B cells within the two compartments are clonally related and derived from a common precursor. Thus a subset of B cells that are activated in the T cell rich area by a T dependent antigen and form foci, migrate into the follicle to initiate the GC reaction (Jacob and Kelsoe, 1992). The cells within the two compartments also differ phenotypically and can be distinguished on the basis of binding to the plant lectin – PNA (Rose et al, 1980). Recent evidences suggest that some distinction exists between the two subsets of cells (Han et al., 1997; Kosco-Vilbois et al., 1997). For example, the overall clonal heterogeneity of GC B cells is markedly decreased in comparision with those 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 have been shown to be subjected to a stringent selection process that operates at the pre GC phase.

Using a 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 (HQLDPAGANSTNPDDGIEKKIAKMEKASSVFNVVNS). This peptide was a chimeric peptide in which residues 1-15 (segment PS1) represent 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) corresponds 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 is 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 of the peptide (Agarwal et al., 1996). A subsequent class switch to the IgG isotype, however, was accompanied by a stringent selection in favour of the cells specific for the tetrapeptide sequence DPAF (sequence 4-7). This monospecificity for the DPAF epitope was retained throughout the primary and secondary response (Agarwal et al., 1996). This selection process was shown to occur prior to initiation of GC reaction and was dependent upon the availability of Ag specific Th cells and the ability of Ag activated B cells to recruit them (Agarwal and Rao, 1997). Further, it was demonstrated that, while the Ag-driven recruitment of appropriate preimmune B cells for IgM Ab production could be achieved in the presence of a marginal level of T cell help, subsequent generation of IgG response required significantly higher numbers of Ag-specific Th cells (Agarwal and Rao, 1997). These results suggested that the step involving class switch was rate limiting, and involved a stringent selection for only a fraction of the antigen stimulated B cells. Importantly, an extension of these studies revealed that low affinity interactions between the BCR and Ag (Ka ≥10^4 M^-1) were sufficient to drive resting B cell activation (Nakra et al., 2000). Thus, in other words, even very low affinity interactions of antigen with preimmune B cells appears to be capable of inducing primary IgM response. These collective results, therefore, suggested that antigen dependent activation of naïve, resting B cells is a facile process requiring low activation thresholds. Both limiting frequency of Th cells, and low affinity of antigen for the BCR 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 for Ag.

It was these earlier studies from our laboratory that raised the possibility that the affinity of antigen for the BCR may constitute at least one regulatory parameter that defines the extent of GC formation in a primary humoral response. Therefore, the present study was undertaken to identify some of the underlying mechanisms by which affinity of BCR for antigen may regulate the functional status of preimmune B cells. To address this question, we first developed an ex vivo experimental system where resting B cells
were purified from the BALB/c IgHa mice and treated with F(ab)_2 fragment of anti-IgD (Results I). Following stimulation, B cells were shown to attain two distinct phenotypic states of activation. They either selectively expressed markers characteristic of an activation phenotype (i.e. slgD^b, I-A^hi, CD86^hi), or, in addition to these markers, they also displayed increased expression of markers typical of the GC phenotypic state (i.e. PNA-R^hi, CD24^hi, GL7^hi). The functional relevance of these two phenotypic states could be established by our demonstration (Results I) that development of GCs from these cells was observed only upon their expression of the GC phenotype. For that anti-IgD stimulated B cells from BALB/c Igir mice were loaded with T-cell epitope CT3 and subsequently transferred into the CT3 primed BALB/c Iglt mice. Stimulation of these B cells with anti-IgD gives them the potential to seed GC although the later process was strictly dependent upon the T cell help (Results I).

The majority of antigen receptor expressed on the surface of resting B cells is of the IgD isotype (Abney et al., 1978). The amount of slgD exceeds that of slgM by about 10 fold (Havran et al.,1984). Although both slgM and slgD are considered to be functionally similar, it is the slgD that serves as the principal sensor for antigen (Brink et al., 1995). In addition to its increased surface density, slgD also represents an efficient antigen binding receptor due to its unique structural flexibility that is provided by an extended hinge region (Blattner and Tucker, 1984). It has been proposed that slgD might function as auxiliary receptor that optimizes the efficient recruitment of antigen activated B cells into T-dependent humoral responses. This was suggested from experiments in IgD knockout mice where both antibody production and affinity maturation was found to be delayed (Roes and Rajewsky, 1993). Thus, by accelerating affinity maturation in the primary humoral response, IgD is thought to play critical role in the defense against pathogens.

Antigen interaction with the cognate receptor on B cells leads to initiation of diverse intracellular signaling pathways and subsequent recruitment of second messengers (Campbell, 1998; Kurosaki, 2002; Benschop and Cambier, 1999). As discussed in Chapter I, it is the recruitment of second messengers that eventually leads to transcription factor activation and gene expression. In this study, by using a variety of pharmacological inhibitors, we have identified the key second messengers that mediate
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the surface expression of activated and GC markers upon treatment of resting B cells with anti-IgD. For this the effect of EGTA, Cal C and H-89 were analyzed. The acquisition of both the activated and the GC phenotype could be inhibited in the presence of EGTA and Cal C, whereas H-89 selectively inhibited surface induction of the GC markers. While the activation of the phosphoinositol signaling pathway could alone drive expression of the activation related markers, cooperative interactions from cAMP dependent pathways were required in order to mediate induction of the GC markers. The extent of crosstalk between these two pathways i.e. phosphoinositol and cAMP dependent - regulate the activation status of preimmune B cells. Interestingly, these two pathways could also be shown to emanate from two distinct isoforms of the sIgD receptor (Results I).

A highlight of the present study is the identification a novel isoform of surface IgD in primary murine resting B cells (Results I). This isoform could be distinguished from its transmembrane counterpart by the fact that its surface expression was mediated by a glycosylphosphotidylinositol anchor. Interestingly, this subset of receptor was found to constitute a minority population, representing only 4-5% of total sIgD pool, and was constitutively localized within the raft domains of the plasma membrane. In addition to its mode of expression, the GPI-linked sIgD could also be distinguished from the transmembrane-sIgD by its ability to induce cAMP - but not the phosphoinositol dependent pathways. Recruitment of the latter pathway was specific for the transmembrane-sIgD. Thus, by recruiting cAMP dependent pathways the GPI-linked sIgD receptor isoform appears to play a critical role during the acquisition of the GC phenotype by resting B cells (Results I).

A variety of other stimuli may also activate either the phosphoinositol or cAMP pathway in B cells, the relative timing at which these two pathways are initiated appears to regulate the biological outcome (Pollock et al., 1991, Natarajan et al., 2001). Asynchronous activation of the two pathways has been shown to produce suppressive effects (Muthuswamy et al., 1991). For positive cooperativity between these two pathways, they need to be activated simultaneously (Natarajan et al., 2001; Cohen and Rothstein, 1989). This cooperativity between the PLCγ and cAMP dependent pathways has also been observed in B cells stimulated simultaneously with anti-IgM and CD54.
The slgM mediated phosphoinositol pathway interacted in a cooperative manner with the CD54 dependent cAMP pathways resultes in the increased activation of CREB in B cells. Thus, although, anti-IgM treatment of B cells does not induce cAMP, slgM mediated signaling nonetheless appears to be sensitive to cAMP-dependent pathways (Natarajan et al., 2001). It is, therefore, possible that by mobilizing cAMP, the GPI-linked slgD may additionally function to enhance slgM -dependent signaling in resting B cells.

The potential for regulation by the two, biochemically distinct, slgD isoform is maximally exploited by resting B cells by ensuring that they are unequally represented on the cell surface. As shown in Results I, the transmembrane form, constituting the overwhelmingly predominant form, represents more than 95% of the total slgD pool. Our results suggests that it is this unequal distribution that facilitates the graded responsiveness of primary B cells. Antigens that either bind to the BCR with higher affinity, or are present in high concentrations would engage both isoforms of receptor thereby ensuring induction of the GC phenotype. On the other hand, antigens that either bind with lower affinity or are present in limiting concentrations are expected to only partially occupy the slgD receptor. Given the predominance of the transmembrane slgD over GPI-slgD we anticipate that this will represent a situation when only the former subset is engaged, thus leading to the selective induction of an activated phenotype. Thus, this segregation of the receptor isoforms permits preimmune B cells to regulate the extent of its activation depending upon the strength of the antigenic stimulus experienced, and thereby modulating the humoral response.

The above results were also be functionally validated by our subsequent results in Results II which demonstrated that differential BCR engagement indeed regulates between the activation versus the GC phenotypic state. We have shown that the two phenotypic states of antigen activated B cells were distinguishable on the basis of the rates at which they are induced, and in their requirement for distinct activation thresholds. While the activated phenotype could be attained within 24 hrs of stimulation, generation of GC phenotype follow delayed kinetics and optimal expression was only observed after extended culture periods for at least 60 hrs. Whether an activated or GC phenotype will be formed was also governed by the triggering thresholds. While an activated phenotype could be achieved by a short duration (30 min) of receptor triggering, an extended
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receptor engagement of at least 36 hrs was required for both the induction of the GC phenotype and conferring of the GC seeding potential (Results II). We interpret this to indicate that the duration for which an antigen is available in the host will play an important role in defining the extent to which activation of B cells have been achieved. Thus, from a physiological point of view, the in vivo stability of antigen in the host is likely to influence the outcome of a humoral response.

In addition to stability of antigen, the concentration at which this antigen is available was also found to be critical in determining whether an activated or a GC phenotype was obtained. At limiting concentrations the anti-IgD could only drive the activation of B cells whereas, for the generation of an effective GC response, higher doses of the stimulant were required. These findings collectively suggest that the acquisition of an activated versus the GC phenotype involves distinct activation thresholds both in terms of the concentration of antigen and the BCR-triggering times required (Results II). Further, the distinction observed in their activation threshold correlates well with the observed differences in the intracellular signals that are required for these effects. While the activation phenotype was dependent solely upon the phosphoinositol – signaling pathways, co-operative contributions from the cAMP-dependent pathway was also needed to generate the GC phenotype. As already discussed, cAMP pathways emanate from GPI-linked slgD that constitutes less than 5% of the total slgD pool. Thus, the activation of this isoform of receptor will only occur in the presence of high concentrations of antigen, or, antigens that bind the BCR with high affinity. Therefore, by defining the extent of crosstalk, intelligent decision-making is achieved in order to appropriately modulate the response of individual resting B cells, depending upon the strength of the antigenic stimulus that they experience.

Although the attainment of a germinal center phenotype was shown to be controlled by BCR-dependent parameters the formation of GCs, however, represents a T-dependent phenomenon. This was also confirmed in our adoptive transfer experiments where, although anti-IgD treatment of B cells conferred upon them the potential to seed GC, development of GCs from these B cells nonetheless still required T cell help. Therefore we also examined whether T cell help can exert additional regulatory effects on antigen activated B cells. Although a cognate engagement of T by B cells involves a
multitude of cell surface interactions, from practical considerations, we chose only three of the molecules expressed by B cells. These molecules were CD54, CD40 and I-A. The results described in Results II demonstrate that crosslinking of these surface molecules could potentiate the effects of limiting concentrations of anti-IgD on resting B cells. Thus, it is likely that the activation threshold barrier imposed by low receptor occupancy can be overcome by secondary stimuli that are contributed by T cells during a cognate engagement. Importantly, a combination of all three antibodies – anti-I-A, anti-CD40, and anti-CD54 – was required to achieve this. This suggests that it is the cooperative contributions from the multiplicity of interactions, established at the B-T interface, that is capable of overcoming the activation threshold barrier inherent to BCR-dependent pathways.

The above results therefore suggest that, antigens that are unable to induce efficient humoral responses at low concentration on their own, can still overcome the barrier of activation threshold by recruiting help from antigen primed T cells. However, the affinity of preimmune B cells for antigen also plays crucial role in defining their ability to recruit T cell help. Further, the GC seeding competency of primary B cells is established within 48 hrs of primary immunization, therefore the availability of primed T cells in early stages of activation may be essential. Thus, antigen can only overcome BCR threshold if it can rapidly prime T cells with a very high efficiency. In other words, the balance between the efficiency with which an antigen can bind to BCR, and that with which it can prime T cells, determines the magnitude of the germinal center response. This, in turn, defines the nature and extent of the memory B cell pool that is generated and, thereby, both qualitative and quantitative aspects of the secondary humoral response.